reduced pressure to near dryness to yield a crude white product; this product was collected by filtration, washed well with petroleum ether, and recrystallized from 95% EtOH to afford 0.65 g (30%) of 8: mp 173–175 °C; IR (KBr) 3200 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.9–7.5 (m, 5, arom H), 7.6 (br s, 1, NH), 3.5 (q, 2, CH_2), 1.3 (t, 3, CH_3).

Method B. A solution of 2-acetamido-5-phenyl-1,3,4-thiadiazole[21] (0.2 g, 1 mmol) in THF (15 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (0.08 g, 2 mmol) in THF (15 mL) at 0 °C. The reaction mixture was heated at reflux for 3 h and cooled to 0 °C and the excess $LiAlH_4$ destroyed by the successive dropwise addition of H_2O (1 mL), 15% aqueous NaOH (1.5 mL), and H_2O (3 mL). The mixture was filtered, the filtrate was dried ($MgSO_4$) and evaporated to dryness to afford 0.09 g (40%) of 8, mp 173–174 °C after recrystallization from 95% EtOH. Anal. ($C_{10}H_{11}N_3S$) C, H, N.

Anhydro-2-phenyl-6,8-diethyl-5-hydroxy-7-oxo-1,3,4-thiadiazolo[3,2-*a*]pyrimidinium Hydroxide (3d). Bis(2,4,6-trichlorophenyl)ethylmalonate (0.39 g, 0.8 mmol) and 8 (0.2 g, 0.8 mmol) were heated, neat, at 160 °C until a clear melt resulted (ca. 5 min). The cooled product was triturated with anhydrous Et_2O (20 mL) and collected by filtration. Recrystallization from *i*-PrOH yielded 0.17 g (98%) of 3d as pale yellow crystals: mp 233–235 °C; IR (KBr) 1685, 1645 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.0–7.6 (m, 5, arom H), 4.25 (q, 2, CH_2), 2.6 (q, 2, NCH_2), 1.6 (t, 3 CH_3), 1.1 (t, 3, NCH_2CH_3). Anal. ($C_{15}H_{15}N_3SO_2$) C, H, N.

Biochemical Assay. Inhibition of binding of 1 nM [^3H]cyclohexyladenosine (New England Nuclear Corp.) to A_1-adenosine receptors in rat cerebral cortical membranes was assayed as described.[14] The K_D for [^3H]cyclohexyladenosine was about 1 nM. Inhibition of binding by a range of concentrations of each compound was assessed in triplicate in one to three separate experiments. Inhibition of 2-chloroadenosine-stimulated accumulation of cAMP in [^3H]adenine-labeled guinea pig cerebral cortical slices was determined essentially as described.[14] In addition, 10 μg/mL of adenosine deaminase was present in final incubations to eliminate contributions from endogenous adenosine and 30 μM rolipram [4-[3-(cyclopentyloxy)-4-methoxyphenyl]-2-pyrrolidone, ZK 62711, Schering AG, West Berlin] was present in final incubations to inhibit phosphodiesterases. The EC_{50} of 2-chloroadenosine was approximately 7 μM. Inhibition of the response to 15 μM 2-chloroadenosine by a range of concentrations of each compound was determined in triplicate in one to two separate experiments. K_i values can be calculated from the observed IC_{50} values (Table I) by using the equation: $K_i = IC_{50}/1 + [\text{adenosine analogue}]/K_D$ or EC_{50} of the adenosine analogue.

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Registry No. 1a, 58-55-9; 1b, 5169-95-9; 1c, 31542-62-8; 1d, 31542-68-4; 1e, 58-08-2; 1f, 961-45-5; 2a, 91265-82-6; 2b, 91265-80-4; 2c, 91265-83-7; 2d, 91265-84-8; 2e, 91280-59-0; 2f, 91265-85-9; 2g, 91265-86-0; 3a, 39456-06-9; 3b, 91265-87-1; 3c, 53528-96-4; 3d, 91265-81-5; 3e, 53528-87-3; 3f, 91265-88-2; 3g, 53528-93-1; 3h, 91265-89-3; 4a, 91265-90-6; 4b, 91265-91-7; 4c, 91265-92-8; 4d, 91265-93-9; 5, 91265-76-8; 6, 91265-77-9; 7, 91265-78-0; 8, 91265-79-1; 2-(*n*-propylamino)thiazole, 78508-32-4; bis(2,4,6-trichlorophenyl) *n*-propylmalonate, 77427-41-9; carbon suboxide, 12795-06-1; 2-(ethylamino)-1,3,4-thiadiazole, 13275-68-8; 4-ethyl-3-thiosemicarbizide, 13431-34-0; trimethyl orthobenzoate, 707-07-3; 2-acetamido-5-phenyl-1,3,4-thiadiazole, 28898-88-6; bis(2,4,6-trichlorophenyl) ethylmalonate, 15781-72-3.

Synthesis of a Tricyclic Aphidicolin Analogue That Inhibits DNA Synthesis in Vitro

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We have hypothesized that the biological activity of the antiviral antitumor diterpene aphidicolin requires a specific stereochemical relationship between two rigidly held hydroxyl groups on the α face of the molecule. The complex tetracyclic carbon skeleton is not necessary but appears to serve only as a framework on which to hold the hydroxyls. In support of this theory, we have prepared a simple tricyclic triol analogue (7) whose activity approaches that of the natural product in inhibiting in vitro DNA synthesis.

Aphidicolin (1), a diterpenoid tetrol produced by the mold *Cephalosporium aphidicola* Petch,[2] has provoked wide interest in recent years owing to its striking biological activity; more than 100 publications have appeared in the last 5 years reporting its use in biochemical studies. For example, aphidicolin shows marked activity against herpes virus, both in vitro and in the rabbit eye.[3,4] In addition, aphidicolin possesses considerable antitumor activity in the C6 mouse colon and B16 mouse melanosarcoma screens[5] and has been shown to inhibit the growth of leukemic T- and B-lymphocytes.[6] These biological properties, together with the unusual structure of aphidicolin, have also occasioned much activity among synthetic organic chemists. Six different total syntheses of the natural product have been recorded,[7-12] but there has

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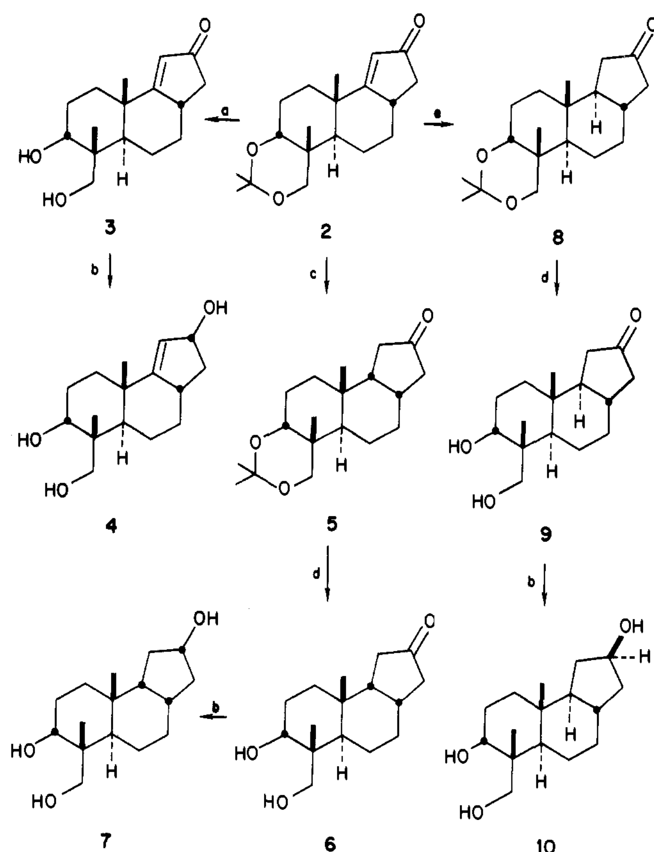
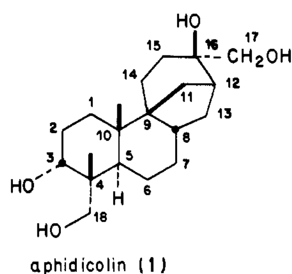
Scheme I. Synthesis of Aphidicolin Analogues[a]



[a] (a) CF_3COOH, CH_2Cl_2, (b) $LiAlH_4$, THF, (c) H_2, Pd/C, CH_3OH, (d) *p*-TsOH, H_2O, CH_3OH, (e) Li, NH_3, THF, *tert*-butyl alcohol, then CrO_3, H_2O, H_2SO_4, acetone.

as yet been only one report of the (microbial) synthesis of aphidicolin analogues.[13]



aphidicolin (1)

Aphidicolin appears to act as a specific reversible inhibitor of DNA polymerase-α.[14-16] Although the reasons for this activity are not clear, molecular models reveal the striking fact that all four of aphidicolin's hydroxyls can very nearly touch the same flat surface, an observation that may well be connected with the biological activity of the molecule. It appears, however, that not all of the hydroxyl groups are required for activity. Preliminary structure–activity studies[3] have indicated that the nonrigidly held hydroxyl groups at C17 and C18 are less important than the rigidly held hydroxyls at C3 and C16. Thus, acetylation of the C17 hydroxyl group causes only a 20% diminution of activity against herpes simplex type 1 virus in human lung cultures, but oxidation of the C3 hydroxyl to a ketone causes a 97% loss of activity.

On the basis of these data and on measurements made from molecular models, we have hypothesized that aphidicolin activity requires the presence of two hydroxyl groups rigidly held on a flat carbon framework at a distance of approximately 6.0 Å. Other hydroxyl groups nearby may also aid in binding to an enzyme but may not be required.

Synthetic intermediates produced in our total synthesis of (±)-aphidicolin[7] provided the opportunity to test our hypothesis. In that synthesis, we developed an efficient method for preparing tricyclic keto acetonide **2**. Starting with **2**, we have now prepared the aphidicolin analogues indicated in Scheme I.

Since our hypothesis requires that an active aphidicolin analogue have two rigidly held hydroxyls on the same face of the molecule at a distance of 6.0 Å, our first thought was to remove the acetonide protecting group from **2** and carry out a reduction of the carbonyl group at C12. We had proved in our aphidicolin synthesis that this reduction occurs from the top (β) face of the enone to provide α-alcohol **4**. Molecular models indicate, however, that **4** is sterically dissimilar to aphidicolin in that the distance between the hydroxyls at C3 and C12 is approximately 7.0 Å rather than 6.0 Å as in aphidicolin. Furthermore, models indicate that the C12 hydroxyl in **4** does not protrude as far below the rough plane of the carbocyclic skeleton as does the C16 hydroxyl in aphidicolin. In keeping with this stereochemical assessment, **4** was found to show no activity in inhibiting in vitro DNA synthesis (see below).

We conclude from these results with analogue **4** that the C12 hydroxyl group must be moved nearer the C3 hydroxyl and be made to protrude farther below the carbocyclic plane if the steric environment in aphidicolin is to be mimicked. Both of these changes were effected by preparing triol **7**. Thus, catalytic hydrogenation of enone **2** over a palladium catalyst gave saturated keto acetonide **5** in near-quantitative yield. The stereochemistry of the reduction was assigned both from examination of molecular models and from literature analogy.[17] Acid treatment of **5** to remove the acetonide protecting group, followed by reduction with $LiAlH_4$, then gave a 9:1 mixture of two epimeric triols. Since it is clear from molecular models that the hydride reduction of **6** must occur from the less hindered β face, we assigned structure **7** to the major isomer.

Triol **7** appears from molecular models to be an almost exact duplicate of aphidicolin with respect to the disposition of its three hydroxyl groups. The intramolecular distance between the hydroxyls at C3 and C12 in **7** is identical with the distance between the C3 and C16 hydroxyls in aphidicolin (6.0 Å), and the protrusion of hydroxyls below the plane of the carbocyclic skeleton is also similar. Thus, we were pleased to find that triol **7** showed 45% of the activity of aphidicolin in inhibiting in vitro DNA synthesis at a concentration of 0.33 μg/mL. Since our material is racemic, it is possible that the enantiomer whose absolute stereochemistry corresponds to aphidicolin is 90% as active. This point would, of course, have to be verified by resolution and individual testing of the two enantiomers.

For comparison with **7**, we also synthesized triol **10**. Reduction of enone **2** with lithium in liquid ammonia gave a saturated keto acetonide that was different from **5** and

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was therefore assigned trans-fused structure **8**. Removal of the acetonide protecting group and reduction with $LiAlH_4$ gave a 7:3 mixture of triols. Since the α face of ketone **9** appears to be less hindered, the major isomer was assigned structure **10**. The C12 hydroxyl group in triol **10** is oriented incorrectly according to our stereochemical hypothesis, however, and **10** was found to be inactive as expected.

Biological Activity. Compounds **3**, **4**, **6**, **7**, **9**, and **10** were assayed for their ability to inhibit DNA synthesis in freshly seeded cell cultures. The method used[18] consists of measuring the uptake of [^{3}H]thymidine in freshly seeded cultures of SV-40-transformed WI-38 human lung fibroblasts. Compounds **3**, **4**, **6**, **9**, and **10** showed no activity in this screen, whereas triol **7** showed a 50% inhibition of [^{3}H]thymidine uptake at a concentration of 0.33 μg/mL. In the same screen, aphidicolin showed 50% inhibition at a concentration of 0.15 μg/mL.

Conclusions. We have provided evidence that the biological activity of aphidicolin requires a specific stereochemical relationship between two rigidly held hydroxyl groups on the α face of the molecule. The complex tetracyclic carbon skeleton is not necessary but appears to serve only as a framework on which to hold the hydroxyls. In support of this theory, we have prepared a simple tricyclic triol analogue (**7**) whose activity approaches that of the natural product in inhibiting in vitro DNA synthesis.

Experimental Section

NMR spectra were recorded on JEOL FX-60 and JEOL FX-100 instruments, with chemical shifts reported in parts per million downfield from internal tetramethylsilane standard. IR spectra were recorded on a Perkin-Elmer 237B instrument, calibrated at the 1601-cm^{-1} polystyrene absorption. Melting points were determined on a Thomas-Hoover Uni-melt apparatus and are uncorrected. All reactions were performed under an atmosphere of dry nitrogen. The phrase "worked up in the usual manner" refers to washing the reaction extract with saturated brine, drying the organic layer with powdered anhydrous sodium sulfate, filtering through a sintered glass funnel, and concentrating the product by solvent removal at the rotary evaporator.

Keto Diol 3. A solution of acetonide **2** (50 mg, 0.164 mmol) in dichloromethane (5 mL) was treated with trifluoroacetic acid (0.25 mL) by dropwise addition at 0 °C. After stirring for 1 h at 0 °C, saturated sodium bicarbonate (5 mL) was added, and the organic phase was worked up in the usual manner. Chromatography of the product on silica gel (10:1:89, hexane/methanol/ether) gave diol **4** as white crystals (40 mg, 91%): mp 154–156 °C; IR ($CHCl_3$) 1701, 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.80 (s, 3 H), 1.22 (s, 3 H), 3.48 (s, 2 H), 3.78 (br s, 1 H), 5.75 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 17.7, 19.5, 20.6, 26.1, 29.5, 35.4, 38.4, 39.4, 40.5, 42.5, 71.0, 75.7, 76.3, 122.0, 192.1, 208.1. Anal. Calcd for $C_{16}H_{22}O_3$: C, 73.23; H, 8.47. Found: C, 73.05; H, 8.75.

Unsaturated Triol 4. A solution of diol **3** (20 mg, 0.076 mmol) in tetrahydrofuran (THF, 10 mL) was added to an ice-cooled suspension of $LiAlH_4$ (20 mg, 0.53 mmol) in THF (10 mL). After stirring for 1 h, several drops of 10% NaOH solution were added, and the reaction was worked up in the usual manner to give triol **4** (20 mg, 98%): IR ($CHCl_3$) 3400 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.74 (s, 3 H), 1.07 (s, 3 H), 3.46 (br s, 2 H), 3.71 (br s, 4 H), 4.83 (br s, 1 H), 5.25 (s, 1 H).

Keto Acetonide 5. Enone **2** (500 mg, 1.64 mmol) was dissolved in methanol (40 mL) and hydrogenated over 100 mg of Pd/C for 9 h at 1-atm pressure. After filtration through Celite, removal of solvent at the rotary evaporator gave keto acetonide **5** (494 mg, 98%) as white crystals. Recrystallization from ether/hexane gave the analytical sample: mp 138–139 °C; IR ($CHCl_3$) 1740 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.73 (s, 3 H), 1.17 (s, 3 H), 1.25 (s, 6 H), 4.44 (dd, 2 H); ^{13}C NMR ($CDCl_3$) δ 17.4, 18.8, 20.0, 22.3, 23.4, 29.4, 31.0, 32.2, 34.2, 34.9, 37.0, 47.5, 51.3, 67.8, 73.2, 97.9, 219.5. Anal. Calcd for $C_{19}H_{30}O_3$: C, 74.44; H, 9.89. Found: C, 74.14; H, 9.93.

Keto Diol 6. Ketone **5** (80 mg, 0.26 mmol) was dissolved in methanol (40 mL), and *p*-toluenesulfonic acid (50 mg) in water (1 mL) was added. After stirring for 24 h at room temperature, solid sodium bicarbonate (100 mg) was added and the mixture was diluted with ether. Workup of the ether extracts in the usual manner gave keto diol **6** as white crystals (50 mg, 72%): mp 127–128 °C; IR ($CHCl_3$) 1740 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.72 (s, 3 H), 1.17 (s, 3 H), 3.38 (br s, 2 H), 3.71 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 17.9, 20.3, 22.5, 26.1, 29.2, 30.8, 32.2, 32.8, 35.0, 37.1, 40.0, 47.6, 51.3, 70.9, 76.7, 220.2. Anal. Calcd for $C_{16}H_{24}O_3$: C, 72.67; H, 9.17. Found: C, 72.91; H, 9.44.

Triol 7. A solution of keto diol **6** (110 mg, 0.417 mmol) in THF (10 mL) was added dropwise to an ice-cooled suspension of $LiAlH_4$ (50 mg, 1.32 mmol) in THF (30 mL). After stirring 0.5 h at 0 °C, several drops of 10% NaOH and several drops of water were added. Workup in the usual manner gave triol **7** as an amorphous solid, which was crystallized twice from ether to provide the pure material (75 mg, 68%): mp 199–201 °C; IR ($CHCl_3$) 3350 cm^{-1}; ^{1}H NMR δ 0.71 (s, 3 H), 1.08 (s, 3 H), 3.41 (br s, 2 H), 3.75 (br s, 2 H); ^{13}C NMR [$(CD_3)_2SO$] δ 17.7, 21.2, 23.1, 25.6, 30.7, 31.0, 34.0, 35.0, 40.4, 42.4, 54.3, 69.6, 71.0, 74.0. Anal. Calcd for $C_{16}H_{26}O_3 \cdot {}^1/_2H_2O$: C, 69.75; H, 9.91. Found: C, 69.97; H, 10.20.

Keto Acetonide 8. A solution of enone **2** (500 mg, 1.64 mmol) and *tert*-butyl alcohol (700 mg) in THF (55 mL) was added to a solution of lithium (210 mg) in liquid ammonia (150 mL). After stirring for 0.5 h at -33 °C, piperylene was added dropwise until the blue color was discharged. The ammonia was allowed to evaporate, and the residue was extracted with ether. Workup in the usual manner gave an oily residue that was dissolved in acetone (20 mL) and cooled to 0 °C. Jones reagent (4.5 mL) was added until the red color persisted, and the mixture was diluted with ether (300 mL). Workup in the usual manner and chromatography on silica gel (35% ether/hexane) gave crystalline keto acetonide **8** (440 mg, 88%): mp 90–94 °C dec; IR ($CHCl_3$) 1750 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.75 (s, 3 H), 0.93 (s, 3 H), 1.42 (s, 6 H), 3.40 (dd, 2 H), 3.68 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 17.3, 19.0, 20.7, 23.4, 32.3, 32.5, 35.5, 37.1, 39.0, 42.2, 46.2, 56.6, 68.1, 73.1, 98.0, 218.1.

Keto Diol 9. A solution of keto acetonide **8** (110 mg, 0.351 mmol) and *p*-toluenesulfonic acid (60 mg) in methanol (55 mL) and water (2 mL) was stirred for 20 h, diluted with ether, and worked up in the usual manner to give keto diol **9** as a solid foam (80 mg, 85%): IR ($CHCl_3$) 1740 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.73 (s, 3 H), 0.93 (s, 3 H), 3.46 (br s, 2 H), 3.73 (br s, 1 H).

Triol 10. A solution of keto diol **9** (110 mg, 0.416 mmol) in THF (10 mL) was added dropwise to an ice-cooled suspension of $LiAlH_4$ (50 mg, 1.32 mmol) in THF (30 mL). After stirring for 0.5 h, several drops of 10% NaOH and several drops water were added. Dilution with ether and workup in the usual manner gave a mixture of epimeric triols. After chromatography on silica gel (1% methanol in ethyl acetate), pure triol **10** was obtained (61 mg, 54%): mp 82–85 °C; IR ($CHCl_3$) 3350 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.69 (s, 3 H), 0.82 (s, 3 H), 3.42 (br s, 2 H), 3.69 (br s, 2 H); ^{13}C NMR [$(CD_3)_2SO$] δ 13.8, 17.4, 21.2, 25.5, 29.3, 32.3, 32.8, 35.0, 35.3, 40.3, 43.0, 56.6, 69.8, 70.1, 73.9. Anal. Calcd for $C_{16}H_{26}O_3 \cdot H_2O$: C, 67.60; H, 10.10. Found: C, 67.74; H, 9.82.

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