Tetrahedron, Vol. 37, Supplement No. 1, pp. 319 to 327, 1981
Printed in Great Britain

0040-4020/81/Suppl. 1 0319-09 $02.00/0
Pergamon Press Ltd.

TOTAL SYNTHESIS OF APHIDICOLIN*

JOHN E. MCMURRY, ALEX ANDRUS, GARY M. KSANDER, JOHN H. MUSSER and MARK A. JOHNSON

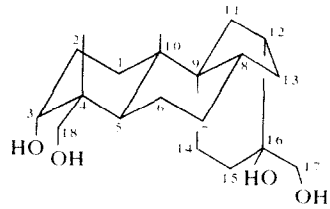
Baker Laboratory, Department of Chemistry, Cornell University, Ithaca, NY 14853‡

(*Received in U.S.A. 7 July* 1980)

Abstract—A total synthesis of the antiviral antitumor diterpenoid, aphidicolin (**1**), is reported. The synthesis starts from the known Δ^4-4,10-dimethyloctalin-3,9-dione (**5**) and requires 15 steps. The two key steps are the Claisen rearrangement of cyclopentenol vinyl ether **16** to unsaturated aldehyde **17**, and the carbonylation of unsaturated tosylate **20** to ketone **21**. The Claisen rearrangement gives largely cyclopentadiene elimination product under most conditions. We found, however, that thermolysis *in the presence of potassium t-pentoxide* gives the desired Claisen product **17** in 60% yield. The second key step is carbonylation of **20** with Collman's reagent, disodium tetracarbonylferrate. This reaction, which has not previously been used in natural products synthesis, takes place in 30% yield, and finishes the construction of the aphidicolin skeleton. (±) Aphidicolin was identical with an authentic sample by IR, ^{1}H and ^{13}C NMR, and GC/MS.

Aphidicolin (**1**) is a diterpenoid tetraol produced by the mold *Cephalosporium aphidicola*, Petch.[1] Rather surprisingly in view of its simple functionality, aphidicolin exhibits striking biological activity. For example, it shows marked activity against herpesvirus, both *in vitro* and in the rabbit eye.[2,3] More recent testing carried out by the National Cancer Institute has revealed that aphidicolin also possesses considerable anti-tumor activity in the C6 mouse colon and B16 mouse melanosarcoma screens.[4]

Although its precise mechanism of action is not known, aphidicolin appears to be a reversible enzyme inhibitor of DNA polymerase-α.[5] Models of aphidicolin reveal the striking fact that all four hydroxyls can very nearly touch the same flat surface. This observation is probably connected with the biological activity of aphidicolin, although it appears that not all of the hydroxyls are required for activity. Structure-activity studies[2] indicate that the non-rigid hydroxyls at C17 and C18 are probably less important than the two rigid hydroxyls at C3 and C16.



1

In view of the biological activity and unique carbon skeleton of aphidicolin, we undertook its total synthesis.[6,7] In this paper, we present the details of our work.

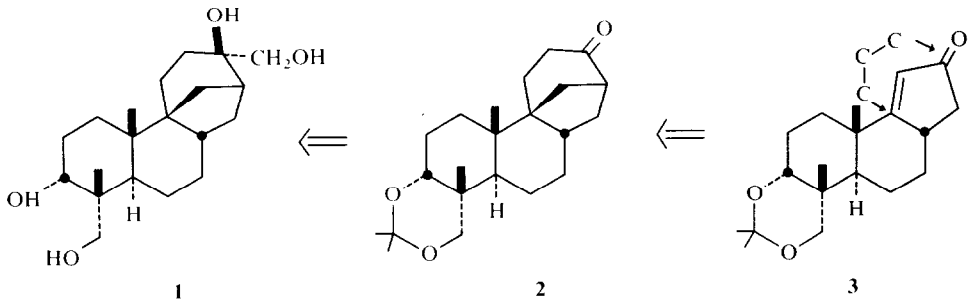
The challenges to synthesis offered by aphidicolin are several. There is, for example, the stereochemical problem presented by the A ring. Although the 3α-hydroxy-4α-hydroxymethyl-4β-methyl substitution pattern seems familiar at first glance, it is in fact unique. No other terpenoid natural product has this stereochemistry. Another and more severe stereochemical problem is that posed by the spiro center, C9. Not only is this spiro center chiral, it is also next to another quaternary carbon, C10. The presence of these two adjacent chiral quaternary centers makes this region of aphidicolin quite crowded and thus a likely source of trouble during any projected synthesis.

In planning our work, we decided to first solve the A-ring problem and then attempt to introduce spiro stereochemistry at C9 in the final stages of the synthesis. It occurred to us that cyclopentenone **3** should be a particularly valuable intermediate, and **3** thus became our initial target. The cyclopentenone ring of **3** will become the cyclopentane ring (carbons 8, 9, 11, 12, 13) of aphidicolin, and the molecule is nicely functionalized to allow addition of the required 3-carbon piece across the ends of the enone system. There is a great variety of ways by which enones can be further elaborated, and cyclopentenone **3** should allow us some flexibility of approach should our first choice of route from **3** → **2** not succeed. Once the 3-carbon piece has been added and keto-acetonide **2** obtained, the final steps from **2** to aphidicolin are known.

We began the synthesis of cyclopentenone **3** starting from 2-methyl-1,3-cyclohexanedione, and Scheme 1 shows our initial work. Robinson annulation with 1-penten-3-one gave the known[8] enedione **5** which could be selectively protected by ketalization of the saturated carbonyl. Reductive hydroxymethylation[9] then gave ketol **8**. Although we were ultimately able to achieve an overall yield of approximately 90% for the transformation from **6** to **8**, great experimental care is required in this step. Reduction of enone **6** with lithium in liquid ammonia, followed by enolate trapping with chlorotrimethylsilane, proceeded smoothly to give **7**. Regeneration of the specific enolate followed by its reaction with formaldehyde, however, must be done under exacting conditions (Experimental). Thus, it is critical that all glassware be washed with aqueous ammonia and thoroughly dried prior to use. It is also critical that the enolate be generated in tetrahydrofuran (THF) solvent, that dry

*Dedicated to the memory of Prof. R. B. Woodward.

‡This work was carried out at the University of California, Santa Cruz, CA 95064, U.S.A.



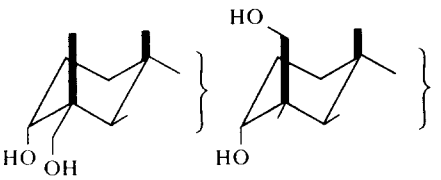
1 2 3

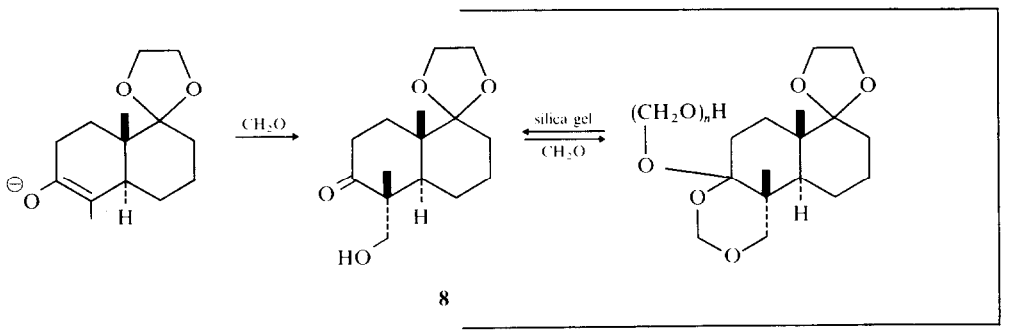
gaseous formaldehyde be introduced, that the formaldehyde be prepared by thermal depolymerization of paraformaldehyde at a temperature not exceeding 140°, and that the gaseous formaldehyde be passed through an ice-cooled trap to remove trace protic impurities (methanol, formic acid) prior to its introduction into the reaction. Even with these precautions, we were initially disappointed to find that the crude product contained no CO group and appeared grossly impure by ^{1}H NMR analysis. We discovered, however, that if the crude hydroxymethylated product is stirred over silica gel in benzene solution, a high yield of pure product **8** is obtained. Presumably, the explanation of this observation is that excess formaldehyde adds to **8** and begins oligomerizing to a host of products. Silica gel treatment unzips the formaldehyde oligomers and regenerates the desired material.

Although crystalline hydroxy ketone **8**, m.p. 105°, could be isolated if desired, it proved more convenient to subject the crude product to reduction with lithium tri-sec-butyl borohydride.[10] This reduction occurred smoothly and in quantitative yield. Interestingly, however, if the reduction was attempted with *potassium* tri-sec-butyl borohydride, only reverse aldolization and loss of formaldehyde occurred.

Diol **9** was next converted into the crystalline keto-acetonide, **10**, by treatment with acetone in dichloromethane in the presence of *p*-toluenesulfonic acid catalyst. Under these conditions, acetonide formation on the 1,3 diol portion, and *trans*-ketalization of the ethylene ketal onto acetone occur concurrently. Keto-acetonide **10** can thus be prepared in 66% overall yield from the known enedione **5**.

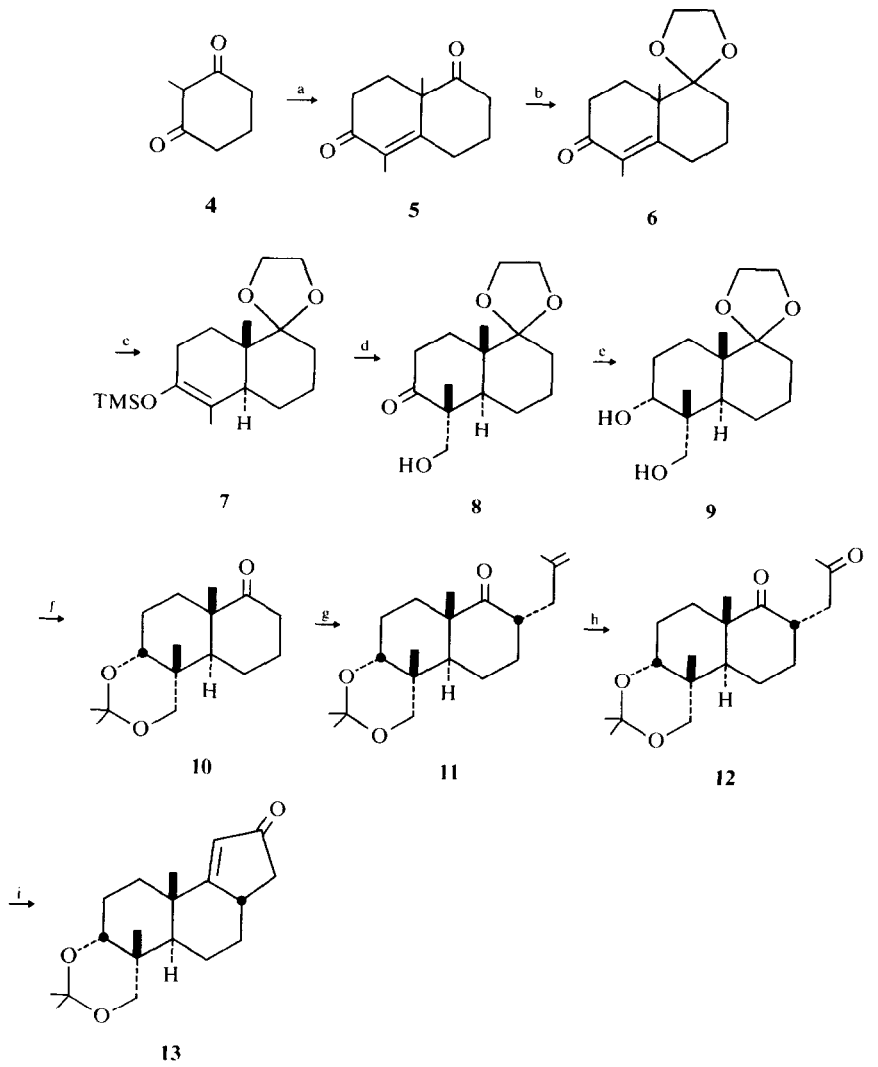
The sequence of reactions from enedione **5** to keto-acetonide **10** provides a single product in high yield. Based on the known[11] stereochemistry of reductive alkylation of substances similar to **5**, and on the known ability of tri-sec-butyl borohydride to produce axial alcohols,[10] we felt that **10** should have the correct stereochemistry at all four chiral centers in the aphidicolin A-ring. Corroboratory evidence comes from two sources, one spectroscopic and one chemical. Spectroscopically, compound **10** is nearly identical to the relevant portion of aphidicolin in both the ^{1}H and ^{13}C NMR spectra. Chemically, we viewed the ready formation of the acetonide grouping as evidence for the correct stereochemistry. Had reductive hydroxymethylation occurred from the β-face and hydride reduction occurred normally, acetonide formation would be quite difficult.





8

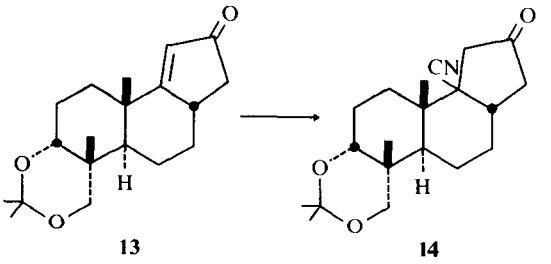
The synthesis of cyclopentenone **3** was completed by alkylation of **10** with methallyl iodide, followed by oxidative cleavage of the side chain double bond and aldol cyclization. The alkylation step proceeded with the desired stereochemistry and generated the fifth necessary chiral center as shown by equilibration experiments (equatorial side chain). Double bond oxidation, **11** → **12**, proceeded without incident using the standard OsO_4–$NaIO_4$ method but the aldol cyclization, **12** → **13**, proved troublesome. Normal protic conditions (NaOH, ethanol) are not effective in this transformation, but we found that sodium t-pentoxide in refluxing benzene provides the key intermediate **13** as white crystals, m.p. 136°, in 88%



Scheme 1. (a) CH_2=$CHCOCH_2CH_3$ pyrrolidine, PhH, 75%. (b) $HOCH_2CH_2OH$, p-TsOH, benzene, 80%. (c) Li, NH_3, THF, and then $(CH_3)_3SiCl$, $(CH_3CH_2)_3N$, 97%. (d) CH_3Li, THF, and then CH_2O. (e) Li(sec-$Bu)_3BH$, THF. (f) CH_3COCH_3, p-TsOH,CH_2Cl_2, 85% from **7**. (g) 1.2 equiv. of LICA, THF, and then methallyl iodide, 89%. (h) Trace of OsO_4, $NaIO_4$, H_2O, dioxane, 86%. (i) NaH, trace of t-amyl alcohol, benzene, reflux, 95%.

yield. All of the transformations to this point are summarized in Scheme 1.

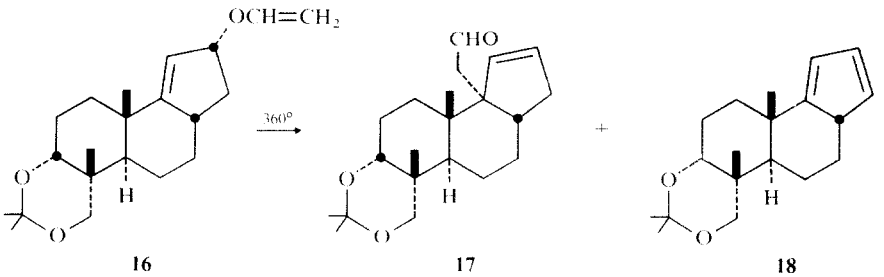
Our initial attempts to use the cyclopentenone functionality for elaboration of the remaining ring centered around the use of conjugate addition reactions. We soon found, however, that the great degree of steric crowding at the enone β-carbon (C9) precluded all chances for success. Thus, sodio-dimethylmalonate, sodionitromethane, lithium diallylcuprate, and lithium divinylcuprate all failed to give any of the desired conjugate addition product on attempted reaction with enone **13**. Conjugate addition of cyanide could be effected in 40% yield using the Nagata procedure,[12] but we were unable to make further use of the nitrile product. Attempted Wittig reaction of keto-nitrile **14** with ethylidenetriphenylphosphorane caused elimination of HCN and simply regenerated enone **13**, while $LiAlH_4$ was without obvious effect on the nitrile function of **14** at 60° in THF.



Stymied in our attempts at *inter*molecular additions to **13**, we next turned to *intra*molecular methods, and our successful route is shown in Scheme 2. Molecular models show clearly that the top face of the carbonyl

group is less hindered to nucleophilic attack than is the bottom face. Thus, $LiAlH_4$ reduction of **13** yields a single crystalline alcohol, m.p. 140–142 , to which we assign structure **15**. This is precisely the stereochemistry needed at the spiro center, C9, if the chirality can be transferred from C12 to C9 by a concerted intramolecular rearrangement. We therefore prepared vinyl ether **16** and began a study of its thermal behavior.

Initial attempts at effecting the Claisen rearrangement **16** → **17** were unsuccessful; a mixture of cyclopentadienes (**18**) resulting from elimination of acetaldehyde is the sole reaction product under most conditions, either gas phase or solution phase. After much experimentation, however, we found that gas phase pyrolysis of allyl vinyl ether **16** at 360 in a nitrogen-swept quartz flow system which had been carefully base-washed and silanized, resulted in a 20% yield of the desired unsaturated aldehyde **17**.

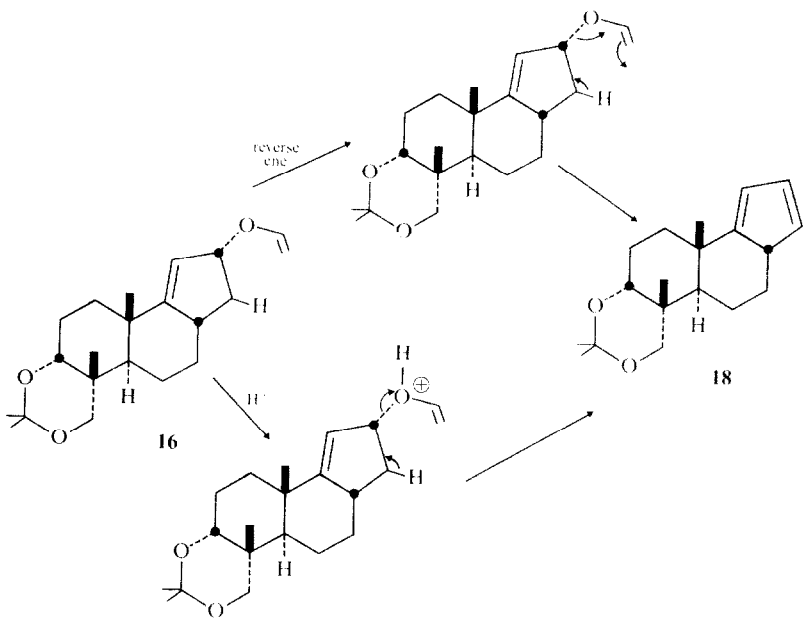


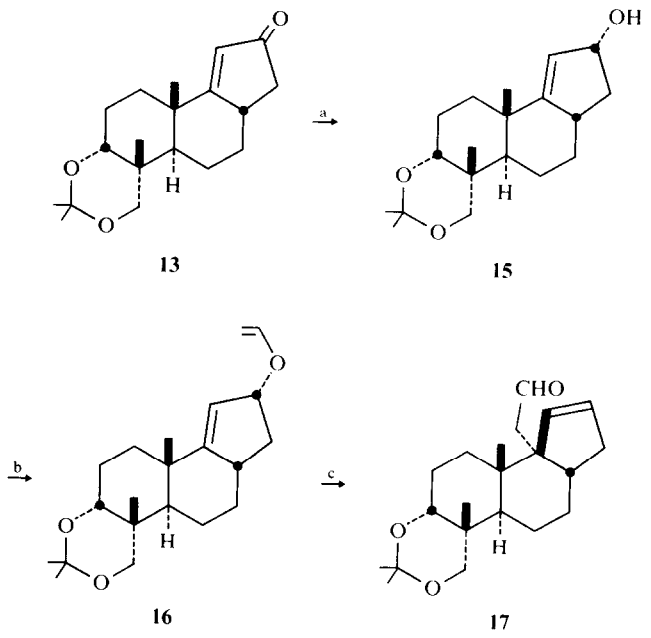
16 **17** **18**

In considering how this yield might be improved, it occurred to us that cyclopentadiene by-product might be formed in either of two ways. Considering the care with which we had washed and neutralized our flow pyrolysis system, it appeared likely to us that cyclopentadiene **18** was simply being formed by a thermal and uncatalyzed reverse ene reaction. Alternatively, however, it was also possible that traces of acid remained on the walls of the flow pyrolysis tube, resulting in an acid-catalyzed elimination of vinyl alcohol *via* a carbocation process.

If the elimination occurs through the pericyclic mechanism, we have no handle by which to control it. If, however, the carbocation mechanism is operative, we should be able to repress it by carrying out the Claisen rearrangement *in the presence of strong base*. We therefore carried out the pyrolysis of **16** for 2 hr at 220 in toluene solution containing a small amount (approx. 0.03% by weight) sodium t-pentoxide, and were pleased to obtain a 60% yield of the desired aldehyde **16**. The yield of the Claisen rearrangement is dramatically increased by carrying out the reaction in the presence of strong base. Although we have not extended our study of this phenomenon to other systems, there is no reason to expect that the effect of base would not be generally applicable in Claisen rearrangements.

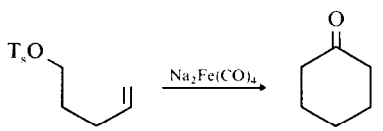
With the Claisen rearrangement successfully concluded, the stereochemical problems of aphidicolin





Scheme 2. (a) $LiAlH_4$, ether, 95%. (b) $CH_3CH_2OCH{=}CH_2$, $Hg(OAc)_2$, 90%. (c) 0.03% NaO-t-pentyl, Toluene, 220°, 60%.

had been solved, and it remained only to knit together the final ring. From the beginning of this work, we had intended to construct this final ring by making use of a novel organometallic reaction which had been heretofore unused in natural products synthesis. Mérour has shown[13] that disodium tetracarbonylferrate ("Collman's reagent") reacts with five- and six-carbon olefinic tosylates to yield cycloalkanones. For example, 1-tosyloxy-5-pentene undergoes carbonylation to yield cyclohexanone. Although the

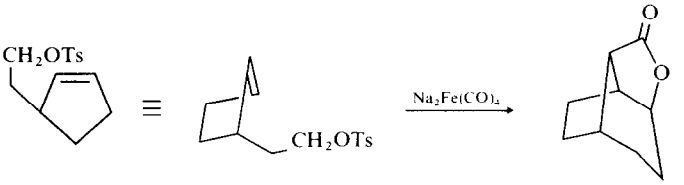


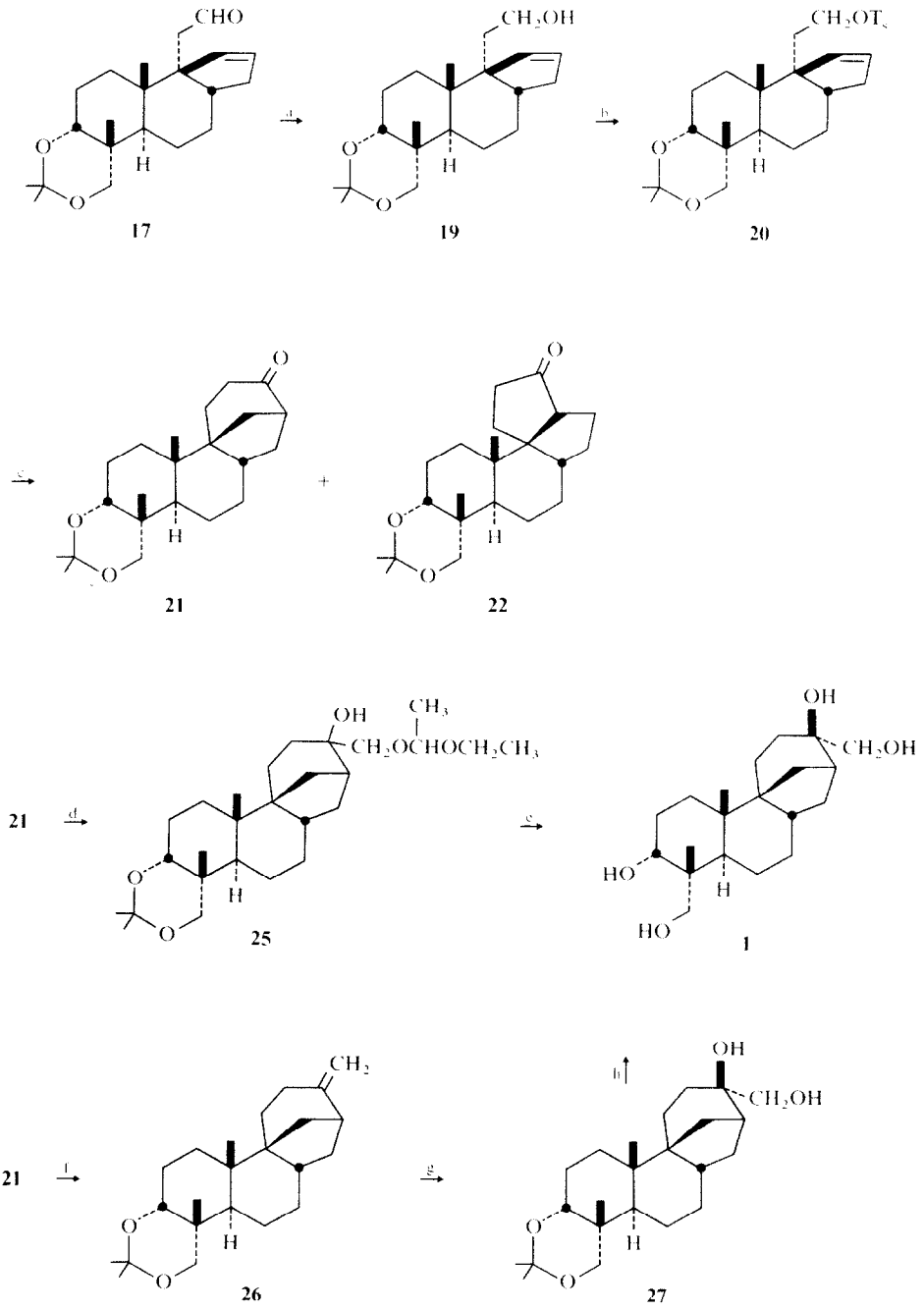
effects of substitution had been little studied, no bicyclo[3.2.1]octanone had been prepared, and no case approaching the complexity of aphidicolin had been attempted, we nevertheless sought to use the Mérour carbonylation reaction for our purposes. The route is shown in Scheme 3.

Aldehyde **17** was reduced with $LiAlH_4$, and alcohol **19** was tosylated. Olefinic tosylate **20** contains the functional groups necessary for Mérour carbonylation to occur, though one might hesitate to predict whether cyclopentanone **22** or cyclohexanone **21** might be formed. In fact, a 55% yield of a 1:1 mixture of **21** and **22** results. This mixture can be readily separated by column chromatography to yield the crystalline keto-acetonide **21**, m.p. 138–139°. The synthetic ketone **21** thus obtained was identical with an authentic sample[14] by IR, ^1H NMR, ^{13}C NMR, and GC/MS.

In retrospect, we were quite fortunate that the carbonylation **20** → **21** occurred as desired, since subsequent work in our laboratory has shown that olefinic tosylate **23**, which might be thought a simple model case, does *not* proceed as desired. Rather, two equivalents of carbon monoxide are incorporated, yielding a substance to which we have assigned structure **24**. While model studies have their proper place in synthesis, results such as this underscore the fact that negative results in a model system must be viewed skeptically.

Completion of the synthesis was accomplished in several ways. The original workers[1] chose a multi-step sequence involving epoxide formation, alkaline hydrolysis, and acetonide formation, at which point aphidicolin bis-acetonide and its C16 epimer could be chromatographically separated. We chose first to look





Scheme 3. (a) $LiAlH_4$, THF, 93%. (b) *p*-TsCl, pyridine, 95%. (c) $Na_2Fe(CO)_4$, N-methylpiperidone, 30%. (d) $LiCH_2OCH(CH_3)OCH_2CH_3$, 63%. (e) H_3O^+. (f) $CH_2{=}P(Ph)_3$. (g) OsO_4, pyridine, then $NaHSO_3$. (h) H_3O^+, 36% from **20**.

at alternate possibilities. Still has demonstrated[15] that 1-ethoxyethoxy-methyllithium adds to ketones to give products which can be hydrolyzed to 1,2-diols. In the present instance, addition of this reagent to keto-acetonide **21**, followed by acidic hydrolysis, gave largely the wrong isomer in contrast to reported results.[7b] Alternatively, Wittig reaction of **21** with methylenetriphenylphosphorane gave olefin **26** which, on treatment with OsO_4 in pyridine, provided diol **27** as an approximately 60:40 mixture. This mixture was hydrolyzed with aqueous acid to aphidicolin **1** and its C16 epimer. These transformations are summarized in Scheme 3.

Separation of the mixture could be accomplished either by fractional crystallization, or by conversion of the mixture into di-acetonides followed by column chromatographic separation and acid hydrolysis. (±) Aphidicolin, m.p. 218–220°, was indistinguishable from an authentic sample[14] by IR, ^1H NMR, ^{13}C NMR, and GC/MS.

EXPERIMENTAL

NMR spectra were obtained in $CDCl_3$ soln on JEOL FX-60 or FX-100 instruments. IR spectra were recorded on a Perkin–Elmer 337, and GC/MS data were recorded on a Finnigan 4000 instrument using a 6 ft OV-17 column. M.ps were obtained on a Hoover–Thomas apparatus and are uncorrected. Combustion analyses were carried out in-house.

Enedione 5. In a N_2 swept, one liter round bottom flask, was dissolved 2-methyl-1,3-cyclohexanedione (34.1 gm, 0.27 moles) in 160 ml benzene. The solution was azeotroped at reflux to remove water with a Dean–Stark apparatus for 1 hr. After cooling, ethyl vinyl ketone (25.0 gm, 0.30 moles) and triethylamine (17 ml, 12.34 gm, 0.122 moles) were added. The soln was refluxed for 5 hr. After cooling, most of the benzene was removed at reduced pressure. Another 100 ml benzene was added and concentration was repeated. A final 160 ml benzene was added, followed by pyrrolidine (5.2 ml, 4.4 gm, 0.062 moles). The dark brown soln was refluxed with a Dean–Stark apparatus for 15 hr. Approximately 3.6 ml of the theoretical amount of 4.5 ml water was separated. After cooling, the soln was diluted with ether and washed with 5% HCl, 5% NaOH and NaCl solns. Drying over $MgSO_4$ and filtration was followed by concentration to give an orange, viscous oil. Kugelrohr distillation gave a pale yellow oil which solidified upon standing in the refrigerator. Recrystallization from hexanes and ether gave white crystals of **5** (38.74 gm, 0.201 moles, 75%): b.p.$_{0.5\,mm}$ 140°, m.p. 38–39 (lit.[8] m.p. 39–40°; NMR: $\delta(CDCl_3)$ 1.80 (s, 3 H), 1.43 (s, 3 H); IR: (neat) 2950, 1710, 1670, 1610 cm^{-1}.

Ketal 6. In a round bottom flask swept with N_2 was placed **5** (10.58 gm, 0.055 moles), ethylene glycol (40 ml, 44 gm, 0.71 moles), 200 ml benzene, and several crystals of *p*-toluenesulfonic acid. The soln was azeotroped at reflux in a Dean–Stark apparatus for 4 hr. Analysis by tlc and IR showed the disappearance of starting material. After cooling, most of the benzene was removed under reduced pressure. Dilution with ether was followed by washing with 5% NaOH and sat NaCl aq. After drying over $MgSO_4$ and filtration, the solvent was removed by rotary evaporation. The viscous yellow oil was dissolved in 5 ml pentane and placed in the refrigerator overnight, which yielded **6** as white crystals (10.35 gm, 0.0438 moles, 80%) m.p. 57–59°; IR: (neat) 2950, 1670, 1615 cm^{-1}; ^1H NMR: $(CDCl_3)$ δ 3.93 (s, 4 H), 1.77 (s, 3 H), 1.33 (s, 3 H); ^{13}C NMR: $(CDCl_3)$ δ 197.8, 159.6, 129.4, 112.2, 64.8, 64.5, 49.8, 38.9, 35.2, 32.2, 27.5, 27.0, 18.1. (Found: C, 71.37; H, 8.63. Calc. for $C_{14}H_{20}O_3$: C, 71.12; H, 8.53%).

Trimethylsilyl enol ether 7. A one liter, 3-neck round bottom flask was fitted with a N_2 inlet, dry ice/acetone condenser, and glass side arm connected to a second one liter flask. From this second flask was distilled 600 ml dry ammonia from Na balls. After distillation was complete, the side arm was replaced with a rubber septum. Li (1.72 gm, 0.248 moles) was added in pieces. After stirring 20 min, 50 ml dry THF was added. A soln of **6** (19.51 gm, 0.0825 moles), t-BuOH (5 ml, 4 gm, 0.054 moles) and 100 ml dry THF was slowly added *via* syringe. After stirring 15 min, 2 ml piperylene was added to quench the excess Li. The soln changed color from deep blue to yellow. Ammonia was distilled off by gentle warming under a flow of N_2. Most of the THF was removed under reduced pressure. Another 100 ml THF was added and the soln was cooled to 0°. A mixture of chlorotrimethylsilane (25 ml, 21.5 gm, 0.198 moles) and triethylamine (25 ml, 18.1 gm, 0.179 moles) was added rapidly *via* syringe. After stirring at 0° for 20 min, the soln was diluted with ether and washed with cold $NaHCO_3$ aq and NaCl aq. Drying over $MgSO_4$ was followed by concentration under reduced pressure and chromatography on silica gel (ether: hexanes, 1:10). After solvent removal, **7** was obtained as a pale, yellow oil (24.86 gm, 0.0804 moles, 97%): IR: (neat) 2950, 1675 cm^{-1}; ^1H NMR: $(CDCl_3)$ δ 3.90 (s, 4 H), 0.95 (s, 3 H), 0.17 (s, 9 H); ^{13}C NMR: (acetone d6) δ 143, 113, 79, 66, 44, 42, 31, 29, 28.5, 25, 24.5, 16, 15, 2 (3).

Keto alcohol 8. All glassware for this reaction was soaked in 20% NH_4OH aq overnight and thoroughly dried in the oven. The setup was assembled hot with a constant stream of N_2. To a 500 ml 3-neck round bottom flask, was added 250 ml of dry THF and MeLi (1.6 M in ether, 20 ml, 0.032 moles). After cooling to 0°, **7** (8.45 gm, 0.027 moles) in 100 ml dry THF was slowly added *via* syringe. Stirring at 0° for 45 min, was followed by cooling to −78°. Gaseous formaldehyde was introduced by depolymerizing paraformaldehyde (dried overnight under vacuum) at 120–145°, and sweeping it with N_2 through a −20° trap and under the surface of the soln. Bubbling in this manner continued for 45 min. Some polymerization of formaldehyde occurred at the inlet. The cold soln was poured into NH_4Cl aq and diluted with ether. The aqueous phase was separated. The organic phase was washed with NaCl aq and dried over $MgSO_4$. Solvent removal gave an oil suitable for the next reaction without additional purification. The product solidified upon standing. Recrystallization from ether/hexanes gave **8**, mp 105°; IR: $(CHCl_3)$ 3450, 2950, 2890, 1690 cm^{-1}; ^1H NMR: $(CDCl_3)$ δ 3.90 (s, 4 H), 3.46, 3.54 (AB quartet, 2 H, J = 5 Hz), 1.30 (s, 3 H), 1.05 (s, 3 H); ^{13}C NMR: $(CDCl_3)$ δ 218.1, 112.5, 66.4, 65.0, 64.6, 52.1, 42.7, 42.2, 35.0, 30.0, 29.4, 22.5, 21.1, 16.7, 16.0. (Found: C, 67.47; H, 9.13. Calc. for $C_{15}H_{24}O_4$: C, 67.13; H, 9.01%).

Diol 9. A dry, one liter round bottom flask was swept with N_2 and filled with 200 ml of dry THF and lithium tri-sec-butyl borohydride (1 M in THF, 57 ml, 0.057 moles). The flask was cooled to −78°. The crude **8** was dissolved in 100 ml dry THF and was slowly added *via* syringe. After stirring at −78° for 1 hr, the temp was raised to 0° for an additional hr. MeOH, 5 ml, was added as a quench. NaOH aq (35 ml, 10%) was added, followed by H_2O_2 aq (80 ml, 30%). Stirring at room temp was continued for 16 hr. After dilution with ether, the soln was washed with water and NaCl aq. Drying over $MgSO_4$ and solvent removal under reduced pressure gave **9** as a viscous oil, suitable for use in the subsequent reaction: IR: $(CHCl_3)$ 3350, 2950 cm^{-1}; ^1H NMR: $(CDCl_3)$ δ 3.93 (s, 4 H), 3.5 (m, 3 H), 1.27 (s, 3 H), 0.75 (s, 3 H).

Keto-acetonide 10. The crude **9** was refluxed with 200 ml CH_2Cl_2, 100 ml acetone, and several crystals *p*-toluenesulfonic acid. After 6 hr, the soln was cooled and most of the solvent was removed by rotary evaporation. Dilution with ether was followed by washing with $NaHCO_3$ aq and NaCl aq. After drying over $MgSO_4$ the solvent was removed to leave a white solid. Compound **10** was recrystallized from ether/hexanes (6.17 gm, 0.0229 moles, 85% from **7**): m.p. 103–104°; IR: $(CHCl_3)$ 2950, 1705 cm^{-1}; ^1H NMR: $(CDCl_3)$ δ 3.63 (t, 1 H, J = 3 Hz), 3.27, 3.65 (AB quartet, 2 H, J = 12 Hz), 1.38 (s, 3 H), 1.35 (s, 3 H), 1.17 (s, 3 H), 0.82 (s, 3 H); ^{13}C NMR: $(CDCl_3)$ δ 215.0, 98.1, 72.6, 68.0, 48.1, 40.5, 37.5, 35.8, 29.3, 26.1, 25.8, 23.1, 20.1, 19.0, 17.8. (Found: C, 72.32; H, 9.95. Calc. for $C_{16}H_{26}O_3$: C, 72.14; H, 9.84%).

Alkylated keto-acetonide 11. Freshly distilled isopropylcyclohexylamine (0.821 gm, 5.81 mmoles) and 10 ml dry THF was placed in a dry, 2 necked round bottom flask swept with N_2. After cooling to 0°, BuLi (2.3 *M* in hexane, 2.3 ml, 5.32 mmoles) was added *via* syringe. Stirring was continued for 20 min before **10** (1.287 gm, 4.84 mmoles) in 8 ml THF was added *via* syringe. The yellow soln was stirred at 0° for 30 min, then cooled to −78° and then warmed to 0° for 2 hr. The soln was poured onto sat NH_4Cl aq and diluted with ether. The aqueous phase was separated. The organic phase was washed with 0.1 N HCl, $NaHCO_3$ aq, and NaCl aq. After drying over $MgSO_4$ and filtration, the solvents were removed under reduced pressure. Chromatography on silica gel gave a white solid after removing the solvent. Recrystallization from ether/hexanes gave crystalline **11** (1.380 gm, 4.30 mmoles, 89%): m.p. 93–96°; IR: $(CHCl_3)$ 2950, 1700, 1650 cm^{-1}; ^1H NMR: $(CDCl_3)$ δ 4.70 (m, 2 H), 3.67 (t, 1 H, J = 2 Hz), 3.30, 3.67 (AB quartet, 2 H, J = 12 Hz), 1.70 (s, 3 H), 1.42 (s, 3 H), 1.38 (s, 3 H), 1.12 (s, 3 H), 0.80 (s, 3 H); ^{13}C NMR: $(CDCl_3)$ δ 213.7, 142.9, 110.9, 97.5, 72.1, 67.3, 47.6, 42.2, 41.0, 37.2, 35.3, 32.5, 29.0, 22.7, 22.0, 19.9, 18.6, 18.4, 17.0.

Diketo-acetonide 12. Compound **11** (2.163 gm, 6.75 mmoles) was dissolved in 38 ml freshly distilled dioxane and 19 ml

water. Osmium tetroxide (0.069 gm, 0.27 mmoles) in 2 ml benzene was added and the soln turned black. After 20 min, sodium metaperiodate (3.608 gm, 16.87 mmoles) was added and the soln was vigorously stirred at room temp for 4 hr. After dilution with ether, the suspension was filtered and washed with sat NaCl aq. Drying over $MgSO_4$, filtration, and solvent removal under reduced pressure gave a black oil, which was chromatographed on silica gel to give an off-white solid. Recrystallization from ether/hexanes gave crystalline **12** (1.88 gm, 5.81 mmoles, 86%): m.p. 104–105°; IR: ($CHCl_3$) 2950, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.63 (t, 1 H, J = 2 Hz), 3.27, 3.60 (AB quartet, 2 H, J = 12.5 Hz), 2.80 (d, 1 H, J = 8 Hz), 2.20 (s, 3 H), 1.38 (s, 3 H), 1.33 (s, 3 H), 1.20 (s, 3 H), 0.82 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 213.3, 206.5, 97.5, 72.0, 67.3, 47.3, 42.5, 40.7, 40.6, 35.1, 31.8, 29.6, 28.6, 25.2, 22.2, 19.5, 18.4 (2), 16.6.

Tricyclic enone **13**. NaH (1.01 gm, 57% oil dispersion, 0.024 moles) was placed in a dry 2-necked round bottom flask, swept with N_2. Three portions of hexanes were used to wash out the oil. The last traces of hexanes were removed under reduced pressure. Benzene (300 ml) and 1 ml of t-pentyl alcohol were added. After stirring for 20 min, **12** (6.283 gm, 0.0195 moles) was added all at once. The mixture was heated at reflux for 2.5 hr. After cooling, the mixture was poured onto cold sat NH_4Cl aq. The aqueous phase was removed and the organic phase was washed with NaCl aq. Drying over $MgSO_4$ filtration, and removal of solvent under reduced pressure gave a yellow semi-solid. By trituration with ether/hexanes, **13** was obtained as white crystals (5.67 gm, 0.0186 moles, 95%): m.p. 136°; IR ($CHCl_3$) 2950, 1700, 1680, 1605 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.70 (s, 1 H), 3.67 (t, 1 H, J = 2 Hz), 3.25, 3.62 (AB quartet, 2 H, J = 12), 2.42 (d, 1 H, J = 6 Hz), 1.38 (s, 3 H), 1.35 (s, 3 H), 1.18 (s, 3 H), 0.78 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 208.5, 193.1, 121.6, 97.7, 72.2, 67.6, 41.8, 40.3, 40.1, 38.5, 37.3, 34.7, 29.2, 28.6, 22.7, 19.6, 19.1, 18.1, 16.5; (Found: C, 74.44; H, 9.23. Calc. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27%).

Allylic alcohol **15**. $LiAlH_4$ (0.125 gm, 3.28 mmoles) was suspended in 80 ml dry ether. The N_2 swept flask was cooled to −20°. **13** (2.00 gm, 6.57 mmoles) in 40 ml dry ether was slowly dripped in *via* syringe. After 30 min, 0.2 ml water, 0.2 ml 15% NaOH, and 0.6 ml water was added. When a white ppt had formed, about a gram of $MgSO_4$ was added. The mixture was filtered through celite. Solvent removal under reduced pressure gave a white solid. The allylic alcohol was recrystallized from ether/hexanes to give **15**; (1.903 gm, 6.21 mmoles, 95%): m.p. 139–152°; IR (KBr) 3300, 2950, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.20 (s, 1 H), 4.75 (br s, 1 H), 3.63 (t, 1 H, J = 2 Hz), 3.23, 3.63 (AB quartet, 2 H, J = 12 Hz), 1.38 (s, 6 H), 1.05 (s, 3 H), 0.75 (s, 3 H); ^{13}C NMR (acetone d6) δ 160.5, 121.2, 76.9, 73.5, 68.6, 42.2, 41.9, 41.6, 37.6, 31.0, 30.5, 29.8 (2), 29.2, 24.4, 21.5, 21.3, 19.5, 17.7. (Found: C, 74.64; H, 9.92. Calc. for $C_{19}H_{30}O_3$: C, 74.47; H, 9.8%).

Allyl vinyl ether **16**. Alcohol **15** (2.606 gm, 8.50 mmoles), freshly recrystallized mercuric acetate (2.980 gm, 9.35 mmoles), and sodium acetate (0.05 gm, 0.61 mmoles) were placed in a dry round bottom flask, into which was distilled 120 ml ethyl vinyl ether (twice distilled from Na). The soln was refluxed gently for 16 hr, then cooled, and most of the solvent was removed under pressure. After dilution with ether, the soln was washed with $NaHCO_3$ aq and NaCl aq. The organic phase was dried over $MgSO_4$, filtered, concentrated and chromatographed rapidly through a short column of silica gel. Solvent removal gave a white solid. Recrystallization from hexanes gave crystalline **16** (2.54 gm, 7.64 mmoles, 90%): m.p. 55–58°; IR ($CHCl_3$) 3075, 2950, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.38 (dd, ABX, 1 H, J = 7, 14 Hz), 5.27 (s, 1 H), 4.88 (t, 1 H, J = 7 Hz), 3.92–4.35 (m, 2 H, J = 1.5 Hz, 7.14), 3.63 (t, 1 H, J = 2 Hz), 3.23, 3.62 (AB quartet, 2 H, J = 12 Hz), 1.38 (s, 6 H), 1.07 (s, 3 H), 0.77 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 162.4, 150.5, 115.3, 98.0, 87.4, 83.2, 72.4, 67.8, 40.5, 37.3, 37.1, 35.6, 35.0, 34.8, 29.5, 28.4, 23.0, 20.3, 18.6, 16.9.

Olefin-aldehyde **17**. A thick walled, 50 ml, high pressure glass tube was soaked in 20% NH_4OH aq overnight and then dried overnight in the oven. Upon cooling, the tube was rinsed twice with several milliliters of bis-trimethylsilyl acetamide. The tube was then charged with **16** (0.280 gm, 0.842 mmoles), about 5 mg sublimed sodium t-pentoxide and 20 ml toluene, which had been passed through alumina and distilled from calcium hydride. The tube was immersed in liquid N_2 and allowed to warm under vacuum. This freeze-pump-thaw cycle was repeated three times. The tube was sealed under vacuum with cooling of the contents. The tube was placed in a preheated oven at 220° for 2 hr. After cooling, the tube was scored and broken open. The clear contents were filtered and concentrated under pressure. The oil was chromatographed on silica gel to give a clear oil. Trituration from ether/hexanes gave the crystalline **17** (0.168 gm, 0.50 mmoles, 60%): m.p. 127°; IR ($CHCl_3$) 3030, 2950, 2750, 1717 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.69 (t, 1 H, J = 3 Hz), 6.17 (d, 1 H, J = 6 Hz), 5.88 (d, 1 H, J = 6 Hz), 3.62 (t, 1 H, J = 2 Hz), 3.20, 3.63 (AB quartet, 2 H, J = 12 Hz), 1.40 (s, 6 H), 1.02 (s, 3 H), 0.70 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 202.3, 136.1, 131.0, 97.0, 72.2, 67.3, 56.8, 44.7, 42.3, 38.2, 36.2, 33.5, 32.5, 28.6, 23.6, 22.7, 20.9, 17.8, 17.2, 15.5. (Found: C, 76.22; H, 9.81; Calc. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70%).

Hydroxy olefin **19**. $LiAlH_4$ (0.082 gm, 2.18 mmoles) was suspended in 10 ml dry THF in a dry, N_2 swept, 2 necked round bottom flask, cooled to −20°. The **17** (0.724 gm, 2.18 mmoles) in 4 ml dry THF was rapidly dripped in *via* syringe. After 30 min, the reaction was quenched with 0.1 ml water, 0.1 ml 15% NaOH aq, and 0.2 ml water. After a white ppt formed, about 0.5 gm $MgSO_4$ was added. The mixture was filtered and solvent was removed under reduced pressure to give a white solid. Recrystallization from ether/hexanes gave crystalline **19** (0.679 gm, 2.03 mmoles, 93%): m.p. 146–148°; IR ($CHCl_3$) 3450, 2950 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.80 (s, collapsed AB, 2 H), 3.1–3.7 (m, 5 H), 2.03 (s, 2 H), 1.42 (s, 6 H), 0.95 (s, 3 H), 0.70 (s, 3 H); ^{13}C NMR ($CHCl_3$) δ 137.3, 131.5, 98.0, 73.5, 68.5, 62.2, 56.5, 45.9, 39.8, 35.4, 34.5, 33.7, 31.7, 29.5, 27.5, 24.6, 23.7, 22.0, 19.1, 18.0, 16.7. (Found: C, 74.92; H, 10.17. Calc. for $C_{21}H_{34}O_3$: C, 75.41; H, 10.25%).

Olefin-tosylate **20**. Freshly recrystallized, dry *p*-toluenesulfonyl chloride (0.122 gm, 0.641 mmoles), **19** (0.195 gm, 0.583 mmoles), pyridine (0.184 gm, 2.33 mmoles), and 3 ml CH_2Cl_2 were stored in a refrigerator at −10° for 36 hr. Dilution with ether was followed by washing with cold 0.1N HCl, $NaHCO_3$ aq, and NaCl aq. After drying over $MgSO_4$, filtration, and solvent removal under reduced pressure, a yellow oil remained. Trituration with hexanes gave colorless crystals of **20** (0.260 gm, 0.554 mmoles, 95%): m.p. 123–125°; IR ($CHCl_3$) 2950, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.68, 7.26 (AB quartet, 2 H), 3.90 (t, 2 H, J = 8 Hz), 3.58 (t, 1 H, J = 2 Hz), 3.17, 3.62 (AB quartet, 2 H, J = 12 Hz), 2.37 (s, 3 H), 1.36 (s, 6 H), 0.87 (s, 3 H), 0.65 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 144.7, 136.3, 133.7, 132.6, 129.8, 127.8, 98.5, 72.8, 70.1, 67.8, 55.8, 45.3, 38.7, 34.5, 33.6, 32.8, 28.9, 26.3 (2), 23.9, 23.1, 21.1, 20.6, 18.2, 17.3, 15.8.

Nor-aphidicolin keto acetonide **21**. The olefin-tosylate **20** (0.124 gm, 0.26 mmoles) was placed in a dry round bottom flask and passed into a glove box. Inside the glove box, 12 ml N-methyl-2-piperidone was distilled from calcium hydride into the flask. Disodium iron tetracarbonyl/dioxane complex (0.308 gm, 0.89 mmoles) was added. The tan mixture turned dark brown as it was stirred inside the glove box for 14 hr. The reaction was quenched with 0.5 ml AcOH, after the flask was removed from the glove box. After dilution with ether, the soln was washed with 0.1N HCl and NaCl aq. The purple soln was passed through alumina, then dried over $MgSO_4$. After filtration, the solvent was removed to give a dark oil. Chromatograohy on silica gel separated the two ketones; **22** eluted first, followed closely by **21**. Each was isolated by solvent removal under reduced pressure and recrystallized from ether/hexanes; **22** (0.023 gm, 0.065 mmoles, 25%): m.p. 139–141°; **21** (0.027 gm, 0.076 mmoles, 30%): m.p. 138–139°. Ketone **21**: IR ($CHCl_3$) 2950, 1710, 1210, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.65 (t, 1 H, J = 2 Hz), 3.23, 3.63 (AB quartet, 2 H, J = 12 Hz), 1.42 (s, 6 H), 1.08 (s, 3 H), 0.75 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 215.0, 97.7, 73.1, 68.1, 48.7, 47.8,

41.0, 39.2, 34.3 (2), 32.9, 32.5, 31.1, 29.5, 26.6, 265.6, 23.5, 21.4, 20.9, 18.7, 16.9, 15.8; (70 eV) 332, 331, 271, 243; *Anal.* Calc. for $C_{22}H_{34}O_3$: C, 76.26; H, 9.89. Found: C, 75.82; H, 9.80. **22**: IR ($CHCl_3$) 2950, 1730, 1450, 1375 cm^{-1}. ^1H NMR ($CHCl_3$) δ 3.63 (t, 1 H, J = 2 Hz), 3.27, 3.65 (AB quartet, 2 H, J = 12 Hz), 1.43 (s, 6 H), 1.00 (s, 3 H), 0.73 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 222.7, 97.6, 72.9, 68.1, 60.6, 52.2, 43.9, 39.2, 36.6, 33.9, 33.2, 29.3, 28.5, 27.7, 26.2, 24.6, 22.8, 22.4, 20.0, 16.5, 15.7.

Methylene-acetonide **26**. Methyltriphenylphosphonium bromide (0.062 gm, 0.173 mmoles) was dried under vacuum for 1 hr. To the 2 necked, round bottom flask was added 2 ml dry ether. BuLi (2.4 *M* in hexane, 0.065 ml, 0.156 mmoles) was added *via* syringe. The yellow liquid was stirred for 2 hr at room temp. **21** (0.020 gm, 0.058 mmoles) in one ml dry ether was added *via* syringe. Stirring under N_2 for 24 hr was continued. The white soln was filtered and solvent removed under reduced pressure to give 24 mg of a white solid. Tlc showed a mixture of triphenylphosphinoxide and **26**. The crude product was used directly in the subsequent reaction. ^1H NMR ($CDCl_3$) δ 4.40 (br s, 2 H), 3.60 (t, 1 H, J = 2 Hz), 3.18, 3.63 (AB quartet, 2 H, J = 12 Hz), 1.40 (s, 6 H), 0.98 (s, 3 H), 0.72 (s, 3 H).

Diol-acetonide **27**. Crude **26** (about 25 mg) was dissolved in 1 ml pyridine. Osmium tetraoxide (0.022 gm, 0.087 mmoles) in 0.3 ml benzene was added. The black soln was stirred at room temp under N_2 for 1 hr. A soln of 0.5 ml pyridine and 0.5 ml 5% $NaHSO_3$ was added. The color of the soln lightened. After 30 min, another 0.5 ml 5% $NaHSO_3$ was added. Stirring continued for another hr to give an orange soln. Dilution with ether was followed by washing with 0.1 N HCl and NaCl aq. The aqueous phase was reextracted with ether. The combined organic phases were dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure to give 29 mg crude, solid **27**; ^1H NMR ($CDCl_3$) δ 3.64 (t, 1 H, J = 2 Hz), 3.57 (m, 2 H), 3.20, 3.60 (AB quartet, 2 H, J = 12 Hz), 1.40 (s, 6 H), 1.16 (s, 3 H), 0.73 (s, 3 H).

Aphidicolin and 16-epi aphidicolin. The crude **27** (29 mg) was dissolved in 3 ml THF, 0.5 ml MeOH, and 1 ml 5% HCl and stirred for 8 hr. The mixture was extracted with six portions of CH_2Cl_2, dried over $MgSO_4$, and filtered. Solvent removal under reduced pressure gave 18 mg of a white solid. Trituration with ether/hexanes/methanol gave crystalline 16-epi aphidicolin (6 mg, 0.018 mmoles, 31% from **21**, 3 steps): m.p. 233–235°. The mother liquor was concentrated and stored in the refrigerator overnight. White crystals were isolated by removing the solvent by micropipet. Thorough drying under vacuum gave **1** (7 mg, 0.021 mmoles, 36% from ketone **20**, 3 steps): m.p. 218–220°; tlc: (ether/hexanes/-methanol: 4/1/0.3, 3 developments), natural aphidicolin, synthetic aphidicolin, R_f = 0.51, 16-epi aphidicolin, R_f = 0.48; aphidicolin: ^1H NMR (pyridine d5) δ 4.8–6.1 (br, 4 H, –OH), 3.87 (br s, 1 H), 3.72 (s, 2 H), 3.58; 3.88 (AB quartet, 2 H, J = 10 Hz), 1.10 (s, 3 H), 0.78 (s, 3 H); MS (70 eV) 334, 333, 320, 308, 307, 290, 289, 275, 271, 260, 259. (Found: C, 71.28; H, 10.01. Calc. for $C_{20}H_{34}O_4$: C, 70.97; H, 10.12%). 16-Epi aphidicolin: ^1H NMR (pyridine d5) δ 4.9–6.1 (br, 4 H, –OH), 3.95 (br s, 1 H), 3.90 (s, 2 H), 3.66, 3.82 (AB quartet, 2 H, J = 10 Hz), 0.99 (s, 3 H), 0.78 (s, 3 H); MS: (70 eV) 334, 333, 320, 308, 307, 290, 289, 275, 271, 260, 259.

Acknowledgements—We thank Dr. Barrie Nesp, Imperial Chemical Industries for his help in supplying comparison samples of aphidicolin and derived materials. This work was supported by the National Institutes of Health through grant AI 14127. The NMR and GC/MS instruments used in this work were partially funded by chemical instrumentation grants from the National Science Foundation. We thank Mr. Eric Papiashvili for expert assistance in carrying out the spectroscopic analyses.

REFERENCES

[1]K. M. Brundret, W. Dalziel, B. Hesp, J. A. J. Jarvis and S. Neidle, *J. Chem. Soc.* Chem. Comm. 1027 (1972); W. Dalziel, B. Hesp, K. M. Stevenson and J. A. J. Jarvis, *Ibid.* Perkin Trans. **1**, 2841 (1973).

[2]R. A. Bucknall, J. Moores, R. Simms and B. Hesp, *Antimicrob. Agents Chemother.* **4**, 294 (1973).

[3]S. Ikegami, T. Taguchi, M. Ohashi, M. Oguro, H. Nagano and Y. Mano, *Nature* **275**, 458 (1978).

[4]J. Douros and M. Suffness, *New Anticancer Drugs* (Edited by S. K. Carter and Y. Sakurai) p. 29. Springer-Verlag, Berlin (1980).

[5]M. Ohashi, T. Taguchi and S. Ikegami, *Biochem. Biophys. Res. Comm.* **82**, 1084 (1978).

[6]A preliminary account of this work has appeared: J. E. McMurry, A. Andrus, G. M. Ksander, J. H. Musser and M. A. Johnson, *J. Am. Chem. Soc.* **101**, 1330 (1979).

[7]Successful syntheses of aphidicolin have also been recorded by two other groups: [a]B. M. Trost, Y. Nishimura and K. Yamamoto, *J. Am. Chem. Soc.* **101**, 1328 (1979); [b]E. J. Corey, M. A. Tius and J. Das, *Ibid.* **102**, 1742 (1980).

[8]Y. Kitahara, A. Yoshikoshi and S. Oida, *Tetrahedron Letters* 1763 (1964).

[9]G. Stork and J. D'Angelo, *J. Am. Chem. Soc.* **96**, 7114 (1974).

[10]H. C. Brown and S. Krishnamurthy, *Ibid.* **94**, 7159 (1972).

[11]R. S. Mathews, S. J. Girgenti and E. A. Folkers, *Chem. Commun.* 708 (1970).

[12]W. Nagata, M. Yoshioka and M. Murakami, *J. Am. Chem. Soc.* **94**, 4644 (1972).

[13]J. Y. Mérour, J. L. Roustan, C. Charrier, J. Collin and J. Benaim, *J. Organomet. Chem.* **51**, C24 (1973).

[14]We thank Dr. Barrie Hesp, Imperial Chemical Industries, for providing this authentic sample.

[15]W. C. Still, *J. Am. Chem. Soc.* **100**, 1481 (1978).