The Structure and Absolute Configuration of the Antibiotic Aphidicolin: a Tetracyclic Diterpenoid Containing a New Ring System

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The structure and absolute configuration of the novel tetracyclic diterpenoid aphidicolin, an antimitotic and antiviral metabolite of *Cephalosporium aphidicola* Petch, have been determined, and a systematic nomenclature is proposed. Possible biosynthetic routes to aphidicolin are discussed. Derivatives of aphidicolin, including possible biogenetic precursors and the C(3)- and C(16)-epimers of the antibiotic have been prepared. The high resolution i.r. hydroxy-frequencies are reported for a number of ring-A 1,3-diols derived from aphidicolin.

CULTURE filtrates from the fungus *Cephalosporium aphidicola* Petch reduce the mitotic rate of mouse 'L' cells growing in tissue culture[1] and also inhibit the growth of *Herpes simplex* type 1 both in cultures of human embryonic lung cells and in the rabbit cornea.[2] We assign structure (1) to the active component, for which we propose the name aphidicolin.[3]

Aphidicolin, $C_{20}H_{34}O_4$, contains neither carbonyl nor olefinic groups and is therefore tetracyclic. The n.m.r. spectra (Table 1) of the antibiotic and its crystalline mono- (2), di- (3), and tri-acetates (4) revealed the presence of two tertiary methyl groups, two primary alcohol groups at quaternary positions, and a secondary alcohol group. Aphidicolin readily and reversibly formed a bisacetonide (20) and is therefore a tetrol. The fourth alcohol group is tertiary and is vicinal to one of the primary alcohol functions since treatment of aphidicolin with periodic acid in pyridine resulted in the loss of the elements of formaldehyde and formation of a ketone (5). The relationship between the remaining primary alcohol, the secondary alcohol, and one of the two tertiary methyl groups was deduced when

† Formerly Brundret.

[1] S. B. Carter, personal communication.

[2] R. A. Bucknall, personal communication.

[3] B.P. Appl. 3280/1971.

oxidation of aphidicolin with chromic acid gave a diketo-acid (21) which contained a methyl ketone and a tertiary methyl group. This assignment was confirmed when mild alkaline hydrolysis of the keto-diacetate (6), produced by chromic acid oxidation of the diacetate (3), gave a norketodiol (7) containing one secondary and one tertiary methyl group.

and phyllocladane were inconsistent with the carbonyl absorption at 1723 cm^{-1} in the i.r. spectrum of the periodate cleavage product (5).

Acetylation of the ketone (5) gave a bisacetate (8) which readily formed the acetal (24). The base peak at *m/e* 99·0442 in the mass spectrum of the acetal (24) was assigned to the ion (25). This fragmentation [5] is

TABLE 1

Chemical shifts (τ values) for protons in aphidicolin and derivatives [a]

Compound	C(3)–H ($W_{\frac{1}{2}}$/Hz)	$-CH_2O$ (J_{AB}/Hz)	C(4)–CH_3, C(10)–CH_3 (J/Hz)	Miscellaneous
(1) [b,c]	6·12 (6)	6·24, 6·34 (q, 11) 6·25, 6·41 (q, 11)	8·97, 9·21	
(2)	6·36 (6·5)	6·06(s) 6·57, 6·67 (q, 11·5)	9·03, 9·31	7·95(s, OAc)
(3)	6·45 (7)	6·09(s) 5·96, 6·12 (q, 11)	9·04, 9·11	7·96(s,[d] 2 × OAc)
(4)	5·22 (6)	6·08(s) 6·04, 6·28 (q, 10)	9·01, 9·02	7·95(s), 8·05(s [d]) (3 × OAc)
(5)	6·35 (7)	6·58, 6·70 (q,[e] 11·5)	8·97, 9·31	
(6)		6·00(s) 6·04(s)	8·96, 9·01	7·93(s), 8·01(s) (2 × OAc)
(7)		6·59, 6·67 (q,[e] 11)	8·86, 9·03 (d, 7)	
(8)	5·14 (6)	6·00, 6·24 (q, 10·5)	8·94, 8·99	8·01(s), 8·03(s) (2 × OAc)
(9)	6·38 (6·5)	6·54, 6·66 (q,[e] 11·5)	9·07, 9·33	
(10)	6·46 (7)	5·94, 6·10 (q, 10·5)	9·09, 9·13	7·97(s, OAc)
(11)		5·98(s)	8·97, 8·98	7·97(s, OAc)
(12)			8·91, 9·03(d, 7)	
(13)	5·22 (6)	5·95(s)	8·89 [d]	6·90(s), 6·93(s) (2 × $-SO_3Me$)
(14)			8·99, 9·16 [d]	
(15)			9·05, 9·16 [d]	5·53, 5·61 ($>C=CH_2$)
(16)	6·33 (6)	6·50, 6·62 (q,[e] 11)	9·00, 9·28	5·52, 5·60 ($>C=CH_2$)
(17) [b]	5·73br	5·75, 6·23 (q, 10) 6·10, 6·19 (q, 10·5)	8·92, 8·93	
(18)	*ca.* 6·4br	6·37, 6·65 (q,[e] 10·5)	8·94, 9·12	
(19) [b]	6·09 (7)	6·04(s) 6·22, 6·38 (q, 10)	9·01, 9·24	
(20)	6·43	6·29, 6·51 (q, 8·25) 6·42, 6·82 (q, 12·5)	9·05, 9·31	8·65(s [f]), 8·70(s) (2 × $OCMe_2O$)
(21)			8·91	7·84(s, Ac)
(24)	5·20		9·02 [d]	8·05(s,[d] 2 × OAc)
(26)	6·32 (6·5)	6·55, 6·65 (q,[e] 11·5)	9·00, 9·31	5·2[m, C(12)–H]
(27)	6·35	6·42, 6·78 (q, 12)	8·99, 9·29	8·62(s,[d] $-OCMe_2O-$)
(28)	6·36	6·36, 6·78 (q, 12)	9·03, 9·29	8·59(s), 8·61(s) ($-OCMe_2O-$)
(29)	6·36	6·36, 6·76 (q, 12·5)	8·91, 9·26	8·6(s,[d] $-OCMe_2O-$)
(32)		6·92, 7·12 (q,[g] 12)	8·93, 8·96	4·25(m, 10, 6, 2) 4·65(m, 10, 3) } $-CH=CH-$
(33)			8·98, 9·08, 9·12	4·55(m, 9, 5, 2) 4·69(m, 9, 3) } $-CH=CH-$
(34)			8·97, 9·13	4·99(m, =CH) 8·32(d, =CMe)
(35)	6·34	6·41, 6·77 (q, 12)	8·94, 9·29	8·61(s,[d] $-OCMe_2O-$)
(36)	6·39	6·38, 6·78 (q, 12)	9·02, 9·28	5·54, 5·62($>C=CH_2$) 8·60(s,[d] $-OCMe_2O-$)
(41)	*ca.* 6·6br	6·56(s) 6·27, 6·49 (q, 8·25)	8·94, 9·03	8·58(s), 8·62(s [d]), 8·68(s) (2 × $-OCMe_2O-$)
(42)	6·37	7·41(s) 6·36, 6·76 (q, 12)	8·99, 9·28	8·62(s [d], $-OCMe_2O-$)
(44)	6·4	6·19, 6·31 (q, 8·25) 6·40, 6·80 (q, 12·5)	9·05, 9·29	8·63(s [d]), 8·64(s), 8·69(s) (2 × $-OCMe_2O-$)

[a] Unless stated otherwise spectra were measured at 100 MHz for solutions in [$^2H_1$]chloroform at ambient temperatures. [b] In [$^2H_5$]pyridine at 220 MHz. [c] At 52°. [d] Integral corresponds to 2 × Me. [e] After addition of [$^2H_2$] water. [f] Integral corresponds to 3 × Me. [g] Signals assigned to $-CH_2S-$ group.

The above features suggested that aphidicolin might be a tetracyclic diterpenoid and a survey of the known tetracyclic diterpenoid types, common oxygenation patterns, and possible biosynthetic pathways [4] led us to consider three possible structures, [(1) without the detailed stereochemistry shown], (22), and (23), for the antibiotic. Corresponding structures based on kaurane

[4] J. R. Hanson, 'The Tetracyclic Diterpenes,' Pergamon Press, Oxford, 1968, p. 114.

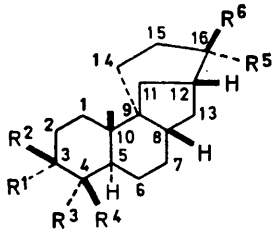
consistent with structures (1) or (22) for aphidicolin but is not readily accommodated by structure (23).

Baeyer–Villiger oxidation [6] of the ketone (5) gave the lactone (26) which was converted into the acetonide

[5] H. Budzikiewicz, C. Djerassi, and D. H. Williams, 'Mass Spectrometry of Organic Compounds,' Holden-Day, San Francisco, 1967, p. 265.

[6] E. J. Corey, N. M. Weinshenker, T. K. Shaaf, and W. Huber, *J. Amer. Chem. Soc.*, 1969, **91**, 5675.

(27). Hydrolysis of the latter with hot aqueous sodium carbonate gave the hydroxy-acid (28) which, when



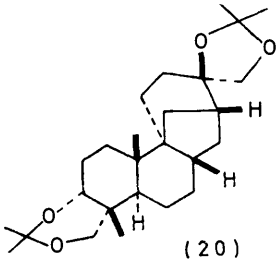
(1) $R^1 = R^6 = OH$, $R^2 = H$, $R^3 = R^5 = CH_2OH$, $R^4 = Me$
(2) $R^1 = R^6 = OH$, $R^2 = H$, $R^3 = CH_2OH$, $R^4 = Me$, $R^5 = CH_2OAc$
(3) $R^1 = R^6 = OH$, $R^2 = H$, $R^3 = R^5 = CH_2OAc$, $R^4 = Me$
(4) $R^1 = R^3 = R^5 = CH_2OAc$, $R^2 = H$, $R^4 = Me$, $R^6 = OH$
(5) $R^1 = OH$, $R^2 = H$, $R^3 = CH_2OH$, $R^4 = Me$, $R_5,R_6 = O$
(6) $R^1,R^2 = O$, $R^3 = R^5 = CH_2OAc$, $R^4 = Me$, $R^6 = OH$
(7) $R^1,R^2 = O$, $R^3 = Me$, $R^4 = H$, $R^5 = CH_2OH$, $R^6 = OH$
(8) $R^1 = OAc$, $R^2 = H$, $R^3 = CH_2OAc$, $R^4 = Me$, $R^5,R^6 = O$
(9) $R^1 = OH$, $R^2 = R^5 = R^6 = H$, $R^3 = CH_2OH$, $R^4 = Me$
(10) $R^1 = OH$, $R^2 = R^5 = R^6 = H$, $R^3 = CH_2OAc$, $R^4 = Me$
(11) $R^1,R^2 = O$, $R^3 = CH_2OAc$, $R^4 = Me$, $R^5 = R^6 = H$
(12) $R^1,R^2 = O$, $R^3 = Me$, $R^4 = R^5 = R^6 = H$
(13) $R^1 = R^3 = MeSO_3$, $R^2 = H$, $R^4 = Me$, R^5, $R^6 = O$
(14) $R^1 = R^2 = H$, $R^3 = R^4 = Me$, $R^5,R^6 = O$
(15) $R^1 = R^2 = H$, $R^3 = R^4 = Me$, $R^5,R^6 = CH_2$
(16) $R^1 = OH$, $R^2 = H$, $R^3 = CH_2OH$, $R^4 = Me$, $R^5,R^6 = CH_2$
(17) $R^1 = H$, $R^2 = R^6 = OH$, $R^3 = R^5 = CH_2OH$, $R^4 = Me$
(18) $R^1 = H$, $R^2 = OH$, $R^3 = CH_2OH$, $R^4 = Me$, $R^5,R^6 = O$
(19) $R^1 = R^5 = OH$, $R^2 = H$, $R^3 = R^6 = CH_2OH$, $R^4 = Me$

oxidised with modified Collins reagent,[7] gave the keto-acid (29). The carbonyl absorption at 1748 cm^{-1} in the i.r. spectrum of the keto-acid (29) [absent in the spectrum of the Wolff–Kishner reduction product (30)] clearly eliminated structure (23) for aphidicolin and supported both structures (1) and (22).

It was clear at this point that a protracted series of transformations would be required to distinguish chemically between structures (1) and (22) and accordingly *X*-ray studies were undertaken to provide an unambiguous structure for aphidicolin. The results of these investigations, which led to the assignment of structure (20) to the bisacetonide of aphidicolin, and hence of structure (1) to aphidicolin, were the subject of a brief communication.[8]

An attempt was made to determine the absolute configuration of the bisacetonide (20) from the anomalous dispersion of Cu-K_α radiation by oxygen. The ratio of oxygen to carbon atoms was less favourable than in any previously reported attempt but preliminary calculations indicated five reflections for which intensity differences due to dispersion might be detectable. Each of these reflections was measured [$\sigma(I) = 0{\cdot}7$—$1{\cdot}0\%$, from counting statistics] in all eight octants, and corrected for absorption by comparing with a neighbouring reflection [$\sigma(I) = 0{\cdot}2$—$0{\cdot}7\%$] for which the dispersion effect was calculated to be small.[9] Values of the corrected quantities $2(I_{hkl} - I_{\bar{h}\bar{k}\bar{l}})/(I_{hkl} + I_{\bar{h}\bar{k}\bar{l}})$ expressed as percentages are compared in Table 2 with values calculated using $\Delta f''_{oxygen} = 0{\cdot}02e$. The results support the assignment of absolute configuration depicted in the structures.

Further evidence for the assignment of absolute configuration was sought by other methods. Wolff–Kishner reduction of the ketone (5) afforded the diol (9) which formed a monoacetate (10). Oxidation of the monoacetate (10), followed by mild alkaline hydrolysis of the keto-acetate (11) yielded the ketone (12). The 4-methyl group in (12) was assigned the α-configuration since prolonged exposure of the ketone (12) to sodium deuterioxide in deuterium oxide resulted in essentially complete incorporation of deuterium at C-4 without



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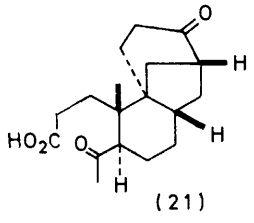

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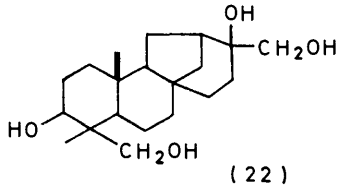

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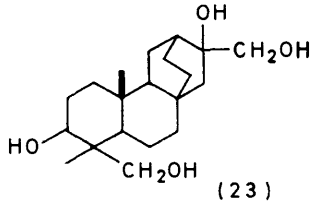

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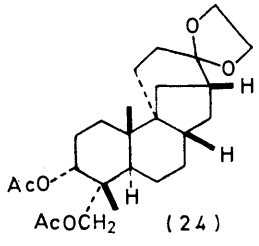

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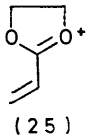

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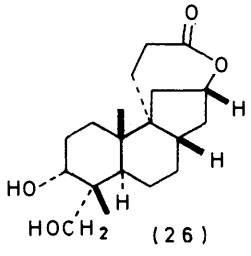

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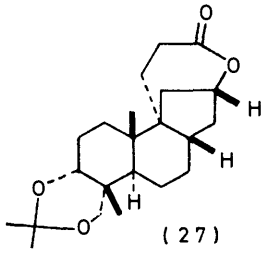

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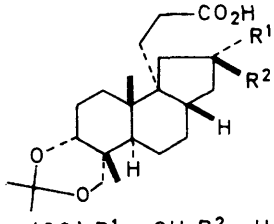


(28) $R^1 = OH, R^2 = H$
(29) $R^1,R^2 = O$
(30) $R^1 = R^2 = H$

[7] R. Ratcliffe and R. Rodehorst, *J. Org. Chem.*, 1970, **35**, 4000.
[8] K. M. Brundret, W. Dalziel, B. Hesp, J. A. J. Jarvis, and S. Neidle, *J.C.S. Chem. Comm.*, 1972, 1027.
[9] D. W. Engel, K. Zechmeister, M. Röhrl, F. Brandl, P. Narayanan, and W. Hoppe, *Nature Phys. Sci.*, 1971, **229**, 28.

epimerisation at this centre. It is known that 4β-methylcholestan-3-one is epimerised by alkali to the thermodynamically more stable 4α-isomer.[10]

The c.d. spectrum of the ketone (12) is given in Table 3. The positive maximum and molecular ellipticity

TABLE 2

Comparison of corrected values $2(I_{hkl} - I_{\bar{h}\bar{k}l})/(I_{hkl} + I_{\bar{h}\bar{k}l})$ with calculated values using $\Delta f'_{oxygen} = 0{\cdot}02e$

hkl	*hkl*/$\bar{h}\bar{k}l$	*hk̄l*/$\bar{h}kl$	$\bar{h}kl$/*hk̄l*	$\bar{h}\bar{k}l$/*hkl*	Mean	Calc.
242	+0·8	+4·9	+3·4	+1·8	+2·7	+3·1
354	+2·6	+1·8	+2·8	+0·3	+1·9	+1·8
361	+2·2	+2·1	+3·2	−0·4	+1·8	+3·3
383	−3·3	−2·0	−5·3	−2·9	−3·4	−2·6
385	+3·2	+3·0	+2·9	+2·1	+2·8	+2·7

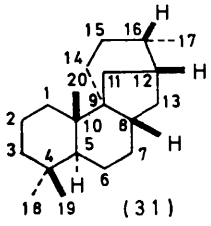
TABLE 3

Circular dichroic spectra of aphidicolin derivatives *

Compound	Molecular ellipticity (degree cm² dmol⁻¹)
(12)	$[\theta]_{325}$ 0; $[\theta]_{287}$ + 4020; $[\theta]_{233}$ 0; $T/_2$ 36 nm
(5)	$[\theta]_{306}$ 0; $[\theta]_{277}$ − 1295; $[\theta]_{224}$ 0; $T/_2$ 35 nm
(14)	$[\theta]_{315}$ 0; $[\theta]_{277}$ − 1335; $[\theta]_{213}$ 0; $T/_2$ 37 nm

* Solutions *ca.* 4 × 10^{-3}M in methanol; 22°; path length 1 cm.

closely resembles the reported value[11] for 17β-hydroxy-4α-methylandrostan-3-one, $[\theta]_{289}$ 3509°. Thus the absolute configuration of the ketone (12) and all other derivatives of aphidicolin is as depicted. The c.d. spectra of two ketones (5) and (14) are also given in Table 3. The negative maxima are consistent with the proposed absolute configuration, the major contributions to the negative maxima presumably arising from carbon atoms C-8 and C-13.



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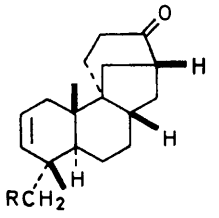
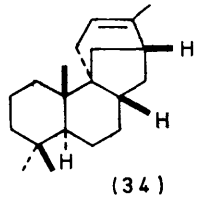


(32) R = SPh
(33) R = H



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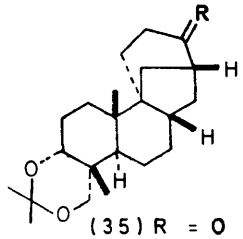
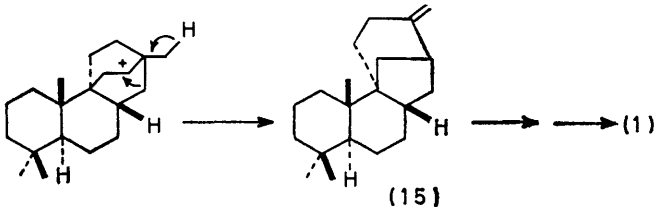


(35) R = O
(36) R = CH_2

Aphidicolin is the first reported member of a new class of tetracyclic diterpenoids. We propose that the hypothetical parent hydrocarbon (31) be named aphidicolane and numbered as shown. Thus aphidicolin is aphidicolane-3α,16,17,18-tetraol. Examples of the skeleton contained in the alternative working structure (22) have been obtained recently by rearrangement of beyerane derivatives.[12,13]

The biosynthetic pathway to aphidicolin remains to be elucidated. However, a comparison of the structures of aphidicolin and other tetracyclic diterpenoids reveals two unusual features which must be accommodated in any proposed biosynthetic pathway to the antibiotic. First, the presence of an 8β-proton in aphidicolin implies the formation of a tricyclic intermediate by a mechanism different from that involved in the pathways to the more common tetratetracyclic diterpenoids[4] and possibly involving a chair–boat cyclisation of geranylgeranyl pyrophosphate (*cf.* pleuromutilin[14]). The second unusual feature is the cyclisation onto C-9, rather than C-8, in a preformed tricyclic intermediate. A 'barrelane' type intermediate, incorporating these two features, may be transformed into aphidicolin by the sequence of unexceptional steps outlined in the Scheme.

Aphidicol-16-ene (15) is a possible biosynthetic precursor of aphidicolin and was prepared as follows. Treatment of the ketone (5) with methanesulphonyl chloride in pyridine gave the dimesylate (13). Protection of the carbonyl group in the latter by acetalisation with ethylene glycol, followed by reaction with benzenethiolate in dimethylformamide and subsequent regeneration of the carbonyl function, afforded the thioether (32) which on desulphurisation with Raney nickel gave the enone (33). Hydrogenation of the enone (33) yielded the ketone (14) which was transformed into aphidicol-16-ene (15) by reaction with triphenylphosphonium methylide. The work-up of the Wittig reaction involved chromatography of the product on silica gel, and resulted in some isomerisation of aphidicol-16-ene to the endocyclic isomer (34). The



(15)

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formation of the ketone (5) by periodate cleavage of aphidicolin, and the sequential protection and regeneration of the carbonyl functions in the dimesylate (13) and the thioether (32), respectively, are each steps in which modification of the bicyclo[3.2.1]octane system could have occurred by Wagner–Meerwein rearrange-

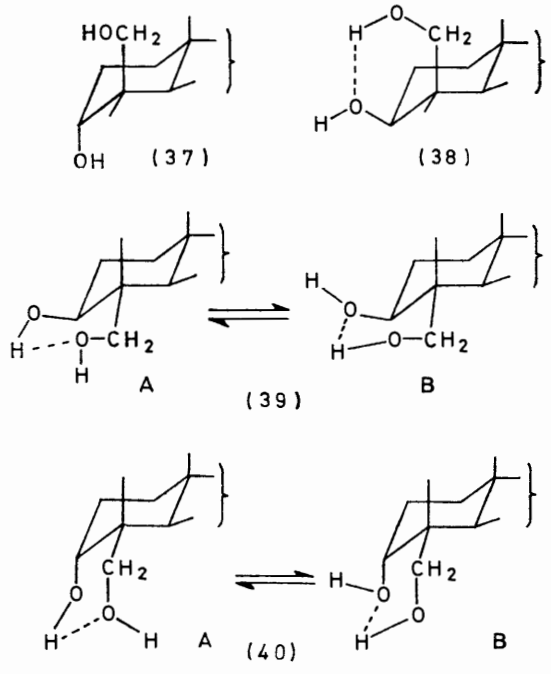
[10] R. B. Turner, R. B. Miller, and J. L. Lin, *J. Amer. Chem. Soc.*, 1968, **90**, 6124.
[11] L. H. Zalkow, R. Hale, K. French, and P. Crabbé, *Tetrahedron*, 1970, **26**, 4947.
[12] K. H. Pegel, L. P. L. Piacenza, L. Phillips, and E. S. Waight, *Chem. Comm.*, 1971, 1346.
[13] M. Laing, P. Sommerville, D. Hanouskova, K. H. Pegel, L. P. L. Piacenza, L. Phillips, and E. S. Waight, *J.C.S. Chem. Comm.*, 1972, 196.
[14] D. Arigoni, *Pure Appl. Chem.*, 1968, **17**, 331.

ments. This possibility was excluded because aphidicolin could be regenerated from the ketone (5) (see below), and because the c.d. spectra of the ketones (5) and (14) were very similar (Table 3).

A preliminary examination of the mycelial products from *C. aphidicola* yielded a hydrocarbon fraction shown by gas chromatography–mass spectroscopy to consist of at least six isomeric hydrocarbons, $C_{20}H_{32}$. Two of the minor components had identical retention times and virtually identical mass spectroscopic fragmentation patterns with those of aphidicol-15- and -16-ene. The mass spectra of the four remaining components were distinguishable from those of the synthetic olefins. Whilst not conclusive, this evidence clearly indicates that aphidicol-16-ene (15) is produced in the mycelium of *C. aphidicola*. The endocyclic isomer (34) may be a metabolic product but could also be produced during work-up. Whether or not aphidicol-16-ene is a precursor of aphidicolin remains to be demonstrated.

The diol (16), itself readily synthesised from the ketone (5) *via* the acetonides (35) and (36), is also a possible biogenetic precursor of aphidicolin. However we did not observe the diol (16) as a fermentation product.

The i.r. spectra of a number of aphidicolin derivatives are of interest in view of the unusual configuration of



their 1,3-diol system. Cole and Müller [15] have reported the hydroxy-stretching frequencies of the 1,3-diols, urs-12-ene-3α,24-diol, urs-12-ene-3β,24-diol, and methyl hederagenin [part structures (37)—(39), respectively]. In this type of compound, primary hydroxy-groups usually absorb at 3640—3642, axial secondary at 3637—3639, and equatorial secondary at 3628—3631 cm^{-1}. The diols (38) and (39) both display broad absorptions due to intramolecular hydrogen bonds and sharper absorptions due to free hydroxy-groups, *e.g.*, the i.r. spectrum of methyl hederagenin (39) displays a broad absorption at 3532 (intramolecular hydrogen bonding) together with a sharper absorption at 3643 (free primary hydroxy-group) and a shoulder at 3628 cm^{-1} (free secondary hydroxy-group). The relative intensities of the last two absorptions show that methyl hederagenin exists mainly as (39A) together with a small proportion of (39B). Cole and Müller did not have a sample of a 1,3-diol containing an axial secondary hydroxy-group and an equatorial hydroxymethyl group but they predicted that this type would show hydrogen bonding, probably as an equilibrium mixture, *i.e.* as in (40A ⇌ 40B).

The aphidicolin derivatives (5), (9), and (16) are examples of Cole and Müller's missing group and we report their hydroxy-frequencies in Table 4. As expected, absorptions due to free and hydrogen bonded hydroxy-groups were found in the spectra of all three compounds but surprisingly the absorptions due to the free hydroxy-groups occurred at frequencies usually assigned to equatorial hydroxy-groups. On the basis of i.r. data alone the secondary hydroxy-group at C-3 in aphidicolin would have been assigned the equatorial configuration. The overall shape ($W_{\frac{1}{2}}$ 6·5 Hz) of the signal due to the C-3 proton in the n.m.r. spectrum of each of the diols (5), (9), and (16) was as expected for an equatorial proton.[16]

TABLE 4

Hydroxy absorption frequencies (cm^{-1}) in aphidicolin derivatives *

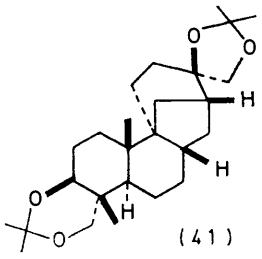
Compound	Free OH	Bonded OH
(5)	3629 3600sh	3545br
(9)	3631 3600sh	3546br
(16)	3631 3600sh	3548br
(18)	3625sh	3534br

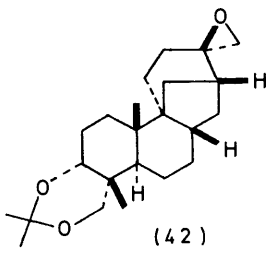
* Spectra, measured for the diols in carbon tetrachloride at at least two dilutions in order to eliminate inteference from intermolecular hydrogen bonding, were calibrated by the water vapour i.r. absorption at 3740 cm^{-1}.

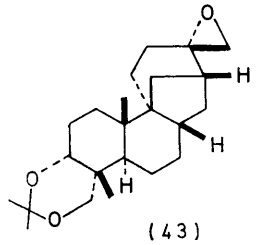
We prepared a derivative of aphidicolin containing the same configuration of hydroxy- and hydroxymethyl groups present in methyl hederagenin (39). Reduction of the keto-diacetate (6) with lithium aluminium hydride gave as major product the tetrol (17) which was isolated as the corresponding bisacetonide (41). Periodate oxidation of the tetrol (17) gave the ketone (18), epimeric at C-3 with the periodate cleavage product (5) of aphidicolin. The frequencies of the i.r. absorptions (Table 4) due to the free and hydrogen-bonded hydroxy-groups in the ketone (18) closely resembled those reported for methyl hederagenin. Unfortunately the broad signals due to the C-3 axial

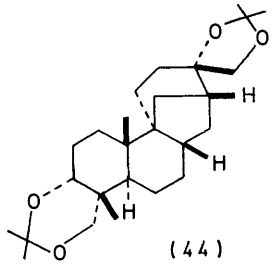
[15] A. R. H. Cole and G. T. A. Müller, *J. Chem. Soc.*, 1959, 1224.
[16] J. E. Bridgeman, P. C. Cherry, A. S. Clegg, J. M. Evans, E. R. H. Jones, A. Kasal, V. Kumar, G. D. Meakins, Y. Morisawa, E. E. Richards, and P. D. Woodgate, *J. Chem. Soc.* (*C*), 1970, 250.

protons in the n.m.r. spectra of the tetraol (17), the bisacetonide (41), and the ketone (18) were not sufficiently well separated from other signals to permit accurate measurement of the half-height widths. However, the n.m.r. spectrum of the triacetate of the tetraol (17) showed a broad multiplet (τ 5·3, $W_{\frac{1}{2}}$ 16·75 Hz)


(41)


(42)


(43)


(44)

well separated from other signals, and clearly due to the C-3 axial proton.[16]

As part of an investigation of structure–activity relationships we also prepared the C-16 epimer of aphidicolin. The acetonide (35) was treated with dimethylsulphoxonium methylide to give a mixture of C-16 epimeric epoxides. The major product (42) of the ylide reaction, obtained by equatorial addition of methylene,[17] could be obtained pure by direct crystallisation. Alkaline hydrolysis of the epoxide (42) followed by treatment of the crude product with acetone and toluene-*p*-sulphonic acid gave aphidicolin bisacetonide (20). The minor component (43) of the ylide reaction product could not be isolated but its presence was inferred when alkaline hydrolysis of the crude mixture, followed by treatment of the product with acetone and toluene-*p*-sulphonic acid, gave a mixture of the epimeric bisacetonides (20) and (44) which were separable by preparative t.l.c. Acidic hydrolysis of the bisacetonide (44) gave the tetraol (19). The structural relationship of the two C-16 epimeric tetraols was confirmed when periodate cleavage of both compounds gave the same ketone (5).

In contrast to the sulphoxonium ylide reaction, which gave a marked preponderance of the epoxide (42), treatment of the ketone (35) with dimethylsulphonium methylide gave approximately equal proportions of the epimeric C-16 epoxides. The n.m.r. spectra of the C-16 epimeric epoxides were very similar but an indication of the proportions of the two epimers present in mixtures was obtained from the integrations of the signals due to the epoxide methylene protons [τ 7·42, in (42); τ 7·36, broader, in (43)].

The biological properties of aphidicolin will be described in detail elsewhere.[18] Aphidicolin (1) was active at 0·2 p.p.m. against *Herpes simplex* type I in cultures of human embryonic lung cells. The monoacetate (2), diacetate (3), tetraol (17), keto-diacetate (6), lactone (26), and tetraol (19) were active at 0·25, 1·6, 4·0, 6·0, 8·0, and 12·5 p.p.m., respectively.[2]

EXPERIMENTAL

Unless stated otherwise i.r. spectra were determined for Nujol mulls. M.p.s were recorded on a Kofler hot-stage apparatus. *X*-Ray data were collected as described elsewhere.[8] Gas–liquid chromatograms were determined with a Pye-Unicam Series 104 instrument using a column 5 ft × 0·25 in (o.d.) of 2% SE30 on GAS-CHROM Q at 160° and with a nitrogen flow rate of 60 ml min^{-1}. Mass spectrometric data were determined in A.E.I. MS9, Hitachi RMU-6E, and LKB 9000 GCMS spectrometers. For gas chromatography-mass spectrometric studies a 5 ft × 0·25 in (o.d.) column of 2% OV-1 on GAS-CHROM Q was utilised; the column temperature was 140° and the carrier gas (helium) flow rate was 30 ml min^{-1}. Optical rotations were determined in a Bendix NPL model 143C automatic polarimeter. Circular dichroic spectra were determined in a Cary 61 spectrometer. Petroleum refers to the fraction of b.p. 60—80°. Silica gel used for column chromatography was Hopkin and Williams MFC. Preparative thin-layer chromatography (p.l.c.) was on Merck silica gel GF 254; layers were 40 × 20 × 0·1 cm and the maximum loading was 150 mg of mixture per plate. Alumina was Woehlm neutral, activity grade III.

Isolation of the Metabolites.—(a) *Aphidicolin* (*aphidicolane-3α,16,17,18-tetraol*) (1). Optimum yields of aphidicolin were obtained when *Cephalosporium aphidicola* Petch [CMI 68, 689 (ii), no. 3490 in our collection] was grown as surface culture for 28 days under a superficial stream of air in glass vessels each containing 1 l of the following medium (g l^{-1}) adjusted to pH 4 : Cerelose (50), glycine (2), magnesium sulphate heptahydrate (1), potassium dihydrogen phosphate (5), potassium chloride (1), and 0·2% (v/v) minor element concentrate.[19] The culture medium (45 l) was filtered, adjusted to pH 6·5, and extracted with chloroform (1 × 15 l, 2 × 9 l). The extract was dried, concentrated to *ca.* 1 l, and set aside to crystallise. The crude aphidicolin (12·3 g) was sufficiently pure for most purposes. A sample (10 g) recrystallised from ethyl acetate gave *needles* (7·03 g), m.p. 227—233°, $[\alpha]_D^{27}$ +12° (*c* 1·0, methanol) (Found: C, 70·8; H, 10·3. $C_{20}H_{34}O_4$ requires C, 71·0; H, 10·1%), $\nu_{max.}$ 3490m, 3410m, 3330s, 1076m, 1046s, 1025s, and 965m cm^{-1}.

(b) *Hydrocarbons.* The powdered, air-dried mycelium from the above fermentation was stirred for 18 h at room temperature with chloroform (4 l). The extracts were concentrated to dryness and the residue was treated for 24 h at room temperature with petroleum (2 l). The petroleum-soluble fraction (62 g) was adsorbed on alumina (300 g) and applied to the top of a column of alumina (700 g) packed in petroleum. Elution with petroleum

[17] E. J. Corey and M. Chaykovsky, *J. Amer. Chem. Soc.*, 1965, **87**, 1353.

[18] R. A. Bucknall, H. Moores, R. Simms, and B. Hesp, *Antimicrobiol. Agents and Chemotherapy*, in the press.

[19] P. W. Brian, P. J. Curtis, and H. G. Hemming, *Trans. Brit. Mycol. Soc.*, 1946, **29**, 173.

(2·5 l) afforded the hydrocarbon fraction (412 mg) as a colourless oil.

Acetylation of Aphidicolin (1).—(a) Acetic anhydride (0·604 g) was added in portions over 48 h to a solution of aphidicolin (2 g) in pyridine (25 ml) at 22°. Water (15 ml) was added and the mixture was left for 3 h. Periodic acid (50% w/w; 17 ml) and water (5 ml) were added and the mixture was stirred for 15 min, then acidified and extracted with ethyl acetate (5 × 20 ml). The extracts were washed with 3N-sodium hydroxide (4 × 15 ml) and water (2 × 50 ml). Evaporation of the solvent gave a solid which was recrystallised twice from ethyl acetate to give *aphidicolin* 17-*acetate* (2) as needles, m.p. 193·5—196° (Found: C, 69·3; H, 9·3. $C_{22}H_{36}O_5$ requires C, 69·4; H, 9·5%), $\nu_{max.}$ 3340m, 1755s, and 1050s cm^{-1}.

(b) A mixture of aphidicolin (3 g) and acetic anhydride (60 ml) in pyridine (90 ml) was stirred for 30 min at 22°, then cooled in ice and treated for 5 h with water (150 ml). Water (200 ml) was added and the mixture was adjusted to pH 2, then extracted with ethyl acetate (5 × 150 ml) to give a gum (3·652 g) which was separated by chromatography on silica gel (200 g). Chloroform eluted fraction A (0·678 g); chloroform–ethyl acetate (40 : 1) followed by chloroform–ethyl acetate (20 : 1) eluted fraction B (0·996 g). Fraction A was separated further by chromatography on silica gel (30 g) to give fraction C (0·299 g). Fractions B and C were combined and recrystallised from ethyl acetate–petroleum to give *aphidicolin* 17,18-*diacetate* (3) (0·825 g) as needles, m.p. 163—166° (Found: C, 67·9; H, 8·9. $C_{24}H_{38}O_6$ requires C, 68·2; H, 9·1%), $\nu_{max.}$ 3420s, 1735s, 1710s, and 1045s cm^{-1}.

(c) A solution of aphidicolin (2 g) and acetic anhydride (40 ml) in pyridine (60 ml) was left for 17 h at 22°, then cooled in ice and treated with water (75 ml) for 4·5 h. Water (200 ml) was added and the mixture was adjusted to pH 2 and extracted with ethyl acetate to give an oil (2·744 g) which was separated by chromatography on silica gel (250 g). Benzene–ethyl acetate (3 : 2) followed by benzene–ethyl acetate (3 : 7) eluted a solid which was recrystallised twice from ether–cyclohexane to give *aphidicolin* 3,17,18-*triacetate* (4) (1·853 g) as needles, m.p. 146—147° (Found: C, 67·2; H, 8·8%; M^+, 464. $C_{26}H_{40}O_7$ requires C, 67·2; H, 8·7%; *M*, 464), $\nu_{max.}$ 3500m, 1745s, 1734s, 1720s, 1270s, 1245s, and 1045m cm^{-1}.

Periodate Oxidation of Aphidicolin (1).—Aphidicolin (11 g) in pyridine (550 ml) and water (220 ml) was treated with periodic acid (50% w/w; 47·6 ml) for 20 min at 22°. The solution was poured into a mixture of ice and sulphuric acid (16·5% v/v; 2 l), then immediately extracted with ethyl acetate (1 × 750, 3 × 500 ml). Removal of the solvent gave 3α,18-*dihydroxy*-17-*noraphidicolan*-16-*one* (5) (8·92 g) as a cream solid, m.p. 150—155°. A sample recrystallised from ethyl acetate–petroleum gave needles, m.p. 155—156° (Found: C, 74·3; H, 9·7. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ (CCl_4) 1723 cm^{-1}.

The Bisacetonide (20).—A solution of aphidicolin (2 g) and toluene-*p*-sulphonic acid (0·5 g) in acetone (200 ml) was boiled under reflux for 25 min, then concentrated to *ca.* 100 ml *in vacuo*. Water (200 ml) was added and the mixture was adjusted to pH 7·5 with saturated sodium hydrogen carbonate. The acetone was evaporated off *in vacuo* and the cream solid (2·42 g) was collected and recrystallised from methanol to give 3α,18;16,17-*bisisopropylidenedioxyaphidicolane* (20) (1·943 g) as rods, m.p. 145—147° (Found: C, 74·2; H, 10·1. $C_{26}H_{42}O_4$ requires C, 74·6; H, 10·1%), $\nu_{max.}$ 1210s, 1100s, 1078s, 1065s, and 865s $cm.^{-1}$.

Hydrolysis of the Bisacetonide (20).—A solution of the bisacetonide (500 mg) and 2N-hydrochloric acid (10 ml) in methanol (200 ml) was left overnight at room temperature. Excess of solid sodium hydrogen carbonate was added and the mixture was stirred for 1 h, then filtered. Removal of the solvent yielded a solid which was washed by repeated trituration with water, then dried and recrystallised from ethyl acetate to give *aphidicolin* (1) (301 mg) as needles, m.p. 224·5—231°.

Chromic Acid Oxidation of Aphidicolin (1).—Chromic acid (15 ml) [prepared from chromium trioxide (1·5 g), conc. sulphuric acid (2·26 g), and water (to 15 ml)] was added dropwise over 40 min to a stirred solution of aphidicolin (500 mg) in glacial acetic acid (20 ml) at room temperature. The mixture was left for 1 h and the excess of chromic acid was destroyed by dropwise addition of ethanol. Extraction with ether afforded a solid (372 mg) which was recrystallised twice from ethyl acetate–petroleum to give 4,16-*dioxo*-17,18-*dinor*-3,4-*secoaphidicolan*-3-*oic acid* (21) (125 mg) as needles, m.p. 173—175° (Found: C, 70·4; H, 8·5%; M^+, 306. $C_{18}H_{26}O_4$ requires C, 70·6; H, 8·5%; *M*, 306), $\nu_{max.}$ 1733s, 1704s, and 1680s cm^{-1}, $\nu_{max.}$ ($CHCl_3$) 1714s cm^{-1}.

Chromic Acid Oxidation of the Diacetate (3).—Chromic acid [2·33 ml of an aqueous solution containing chromium trioxide (0·2 g ml^{-1}) and conc. sulphuric acid (0·18 ml ml^{-1})] was added dropwise over 30 min to a stirred solution of the diacetate (3) (1 g) in acetone (46·5 ml) at 0°. The mixture was left for 20 min at 0° and the excess of chromic acid was destroyed by dropwise addition of ethanol. Water (250 ml) was added and the mixture was extracted with ether to give an oil which was purified by chromatography on silica gel (150 g). Benzene–ethyl acetate (3 : 2), followed by benzene–ethyl acetate (1 : 1) eluted fractions A and B, respectively. Fraction A was separated further by chromatography on silica gel to give fraction C. Fractions B and C were combined (0·689 g.) and recrystallised from ether–cyclohexane to give 16-*hydroxy*-3-*oxoaphidicolane*-17,18-*diyl diacetate* (6) as needles, m.p. 109·5—110·5° (Found: C, 68·5; H, 8·7%; M^+, 420. $C_{24}H_{36}O_6$ requires C, 68·5; H, 8·6%; *M*, 420), $\nu_{max.}$ 3490s, 3410m, 1745s, 1705s, and 1040s cm^{-1}.

Hydrolysis of the Keto-diacetate (6).—A mixture of the keto-diacetate (6) (186 mg), sodium carbonate (300 mg), water (10 ml), and methanol (60 ml) was stirred for 3 days at room temperature, then added to water (100 ml). The methanol was evaporated off *in vacuo* and the aqueous residue was extracted with chloroform (4 × 100 ml). Removal of the solvent and recrystallisation of the product (170 mg) from ethyl acetate–petroleum gave 16,17-*dihydroxy*-19-*noraphidicolan*-3-*one* (7) as felted needles, m.p. 149—150° (Found: C, 74·8; H, 9·7%; M^+, 306. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%; *M*, 306), $\nu_{max.}$ ($CHCl_3$) 1698vs cm^{-1}.

Acetylation of the Ketone (5).—A solution of the ketone (5) (1·0 g) and acetic anhydride (2 ml) in pyridine (3 ml) was stirred for 17 h at room temperature, then poured into ice–water (15 ml) and set aside for 2 h. Water (50 ml) was added and the mixture was adjusted to pH 2 and extracted with ethyl acetate (4 × 25 ml). Removal of the solvent and recrystallisation of the residue (1·15 g) from ethyl acetate–petroleum gave 16-*oxo*-17-*noraphidicolane*-3α,18-*diyl diacetate* (8) as needles, m.p. 178—180°

(Found: C, 70·8; H, 8·7. $C_{23}H_{34}O_5$ requires C, 70·7; H, 8·8%), $\nu_{max.}$ 1735s, 1717s, and 1250s cm^{-1}.

The Acetal (24).—A solution of the keto-diacetate (8) (500 mg), toluene-*p*-sulphonic acid (250 mg), and ethylene glycol (2·5 ml), in benzene (40 ml), was boiled under reflux for 30 min in a Dean–Stark apparatus. Chloroform was added and the mixture was washed with saturated sodium carbonate (50 ml) followed by water (50 ml), then dried (Na_2SO_4). Removal of the solvents gave an oil which was separated by chromatography on silica gel (24 g). Benzene–ether (9 : 1) eluted a solid (282 mg) which was recrystallised from petroleum to give 16,16-*ethylenedioxy*-17-*noraphidicolane*-3α,18-*diyl diacetate* (24) as needles, m.p. 144—146° (Found: C, 68·7; H, 8·7%; M^+, 434·2670. $C_{25}H_{38}O_6$ requires C, 69·1; H, 8·8%; *M*, 434·2668), $\nu_{max.}$ 1735s, 1265s, 1200s, 1115s, 1100s, and 1045s cm^{-1}.

Baeyer–Villiger Oxidation of the Ketone[6] (5).—*m*-Chloroperbenzoic acid (2·125 g) was added over 30 min to a stirred mixture of the ketone (5) (3 g), anhydrous sodium hydrogen carbonate (1·164 g), and methylene chloride (16 ml), at 0°. The mixture was stirred overnight at room temperature, then diluted with methylene chloride (135 ml), and washed with saturated sodium sulphite (2 × 35 ml), saturated sodium hydrogen carbonate (2 × 50 ml), and water (50 ml). Removal of the solvent gave 3α,18-*dihydroxy*-17-*nor*-12,16-*secoaphidicolan*-16,12α-*olactone* (26) (2·86 g) which after four recrystallisations from ethyl acetate–petroleum had m.p. 186—188° (Found: C, 70·7; H, 9·2. $C_{19}H_{30}O_4$ requires C, 70·8; H, 9·4%), $\nu_{max.}$ 3510m, 3490m, 1705s, 1200s, 1190s, 1070m, and 1045s $cm.^{-1}$.

The Acetonide (27).—A solution of the crude lactone (26) (1·836 g) and toluene-*p*-sulphonic acid (1·84 g) in acetone (350 ml) was stirred for 8 days at room temperature, then added to water (350 ml). The solution was neutralised with potassium hydrogen carbonate, concentrated *in vacuo* to remove the bulk of the acetone, then set aside. Recrystallisation of the product from methanol gave 3α,18-*isopropylidenedioxy*-17-*nor*-12,16-*secoaphidolan*-16,12α-*olactone* (27) as long rods, m.p. 168—172° (Found: C, 73·1; H, 9·3. $C_{22}H_{34}O_4$ requires C, 72·9; H, 9·4%), $\nu_{max.}$ 1710vs $cm.^{-1}$.

The Hydroxy-acid (28).—A solution of the lactone (27) (1·55 g) and sodium carbonate (1·36 g) in water (52 ml) was maintained at 100° for 16 h, then cooled, diluted with water (25 ml) and extracted with chloroform (2 × 35 ml). The aqueous layer was acidified and extracted with chloroform to give 12α-*hydroxy*-3α,18-*isopropylidenedioxy*-17-*nor*-12,16-*secoaphidicolan*-16-*oic acid* (28) (1·58 g), m.p. 192—194·5° (sealed capillary) (from ethyl acetate–petroleum) (Found: C, 69·5; H, 9·4. $C_{22}H_{36}O_5$ requires C, 69·4; H, 9·5).

Oxidation of the Hydroxy-acid (28) *with Modified Collins Reagent*.[7]—A solution of chromium trioxide (0·790 g) and pyridine (1·247 g) in methylene chloride (20 ml) was stirred for 15 min at 22°. The hydroxy-acid (28) (0·250 g) in methylene chloride (3 ml) was added and the mixture was stirred for a further 15 min at 22°. The clear solution was decanted from a tarry deposit and the latter was washed with ether (2 × 15 ml). The original supernatant and the washings were combined, washed repeatedly with water until the aqueous washings were colourless, then dried (Na_2SO_4). Evaporation gave a gummy solid (260 mg) which was recrystallised from ether–cyclohexane to give 3α,18-*isopropylidenedioxy*-12-*oxo*-17-*nor*-12,16-*secoaphidicolan*-16-*oic acid* (29) as needles, m.p. 101—105° (Found: C, 71·2; H, 9·7% M^+, 378·2407. $C_{22}H_{34}O_5$,0·5C_6H_{12} requires C, 71·4; H, 9·6%. $C_{22}H_{34}O_5$ requires *M*, 378·2406), $\nu_{max.}$ (CS_2) 1748vs, and 1715s cm^{-1}.

Wolff–Kishner Reduction of the Keto-acid (29).—A mixture of the keto-acid (29) (730 mg), sodium hydroxide (513 mg), hydrazine hydrate (85%; 0·284 ml), and diethylene glycol (8·2 ml), was treated according to the standard procedure,[20] then cooled and added to water (25 ml). The solution was adjusted to pH 2 with 3N-hydrochloric acid and extracted with chloroform (6 × 20 ml). Evaporation of the solvents yielded 3α,18-*isopropylidenedioxy*-17-*nor*-12,16-*secoaphidicolan*-16-*oic acid* (30) (728 mg) which after two recrystallisations from chloroform–petroleum had m.p. 155—157° (Found: C, 72·8; H, 9·9. $C_{22}H_{36}O_4$ requires C, 72·5; H, 10·0%), $\nu_{max.}$ (CS_2) 1714vs, 1200s, 1115m, 1092s, 1002m, and 858m $cm.^{-1}$.

Wolff–Kishner Reduction of the Ketone (5).—A mixture of the ketone (0·887 g), sodium hydroxide (0·504 g), hydrazine hydrate (85%; 0·504 ml), and diethylene glycol (8·05 ml), was treated according to the standard procedure,[20] then cooled and added to water (25 ml). Extraction of the acidified solution with chloroform and recrystallisation of the product from benzene–petroleum afforded 17-*noraphidicolane*-3α,18-*diol* (9), as needles (650 mg), m.p. 156—162°. An analytical specimen had m.p. 160—163° (Found: C, 77·9; H, 11·0%; M^+, 292. $C_{19}H_{32}O_2$ requires C, 78·1; H, 11·0%; *M*, 292).

Acetylation of the Diol (9).—Acetic anhydride (4·21 g) in pyridine (50 ml.) was added in portions during 48 h to a stirred solution of the diol (9) (12·033 g) in pyridine (60 ml) at 22°. The solution was left for a further 74 h at 22°, then treated with ice–water (200 ml) for 30 min. Extraction of the acidified solution with ethyl acetate (4 × 200 ml) afforded a solid (13·1 g) which was separated by chromatography on silica gel (500 g). Chloroform eluted a solid (7·91 g) which on recrystallisation from ethyl acetate yielded 3α-*hydroxy*-17-*noraphicicolan*-18-*yl acetate* (10) (6·05 g) as needles, m.p. 125—134°. An analytical specimen recrystallised from ethyl acetate–petroleum had m.p. 135—137° (Found: C, 75·2; H, 10·3. $C_{21}H_{34}O_3$ requires C, 75·4; H, 10·2%).

Chromic Acid Oxidation of the Monoacetate (10).—Chromic acid [10 ml of an aqueous solution containing chromium trioxide (0·2 g ml^{-1}) and sulphuric acid (0·18 ml ml^{-1})] was added dropwise over 15 min to a stirred solution of the monoacetate (10) (5·434 g) in acetone (200 ml) at 0°. The stirred solution was kept for 30 min at 0°, then diluted with water (2·5 l) and extracted with ethyl acetate to give a gummy solid (5·542 g) which was separated by chromatography on silica gel (220 g). Toluene–chloroform (1 : 4) eluted 3-*oxo*-17-*noraphidicolan*-18-*yl acetate* (11) (4·9 g) as crystals. An analytical specimen recrystallised from petroleum had m.p. 100—102° (Found: C, 76·1; H, 9·7. $C_{21}H_{32}O_3$ requires C, 75·9; H, 9·7%).

Alkaline Hydrolysis of the Keto-acetate (11).—A solution of the keto-acetate (11) (2·3 g), sodium carbonate (4·6 g), and water (161 ml), in methanol (920 ml), was stirred at room temperature for 4 days then poured into water (2 l). Removal of the bulk of the methanol *in vacuo*, followed by extraction of the mixture with ether, gave a solid. A second batch of the keto-acetate (11) (2·6 g was processed similarly and the products were pooled and recrystallised from methanol to give 17,19-*dinoraphidi-*

[20] Huang-Minlon, *J. Amer. Chem. Soc.*, 1949, **71**, 3301.

colan-3-*one* (12) (2·07 g) as needles, m.p. 113—116° (Found: C, 83·3; H, 11·1%; M^+, 260. $C_{18}H_{28}O$ requires C, 83·0; H, 10·8%; M, 260), $\nu_{max.}$ 1700 cm.$^{-1}$. $\nu_{max.}$ (CS_2) 1710s cm.$^{-1}$.

Treatment of the Ketone (12) *with Sodium Hydroxide*.—The ketone (12) (100 mg) in dioxan (1·5 ml) was added to a solution prepared by addition of sodium (25 mg) to dioxan (8 ml) containing water (4 ml). The mixture was stirred under argon for 50 h at room temperature, then added to 0·25N-nitric acid (15 ml), water (75 ml), and ether (75 ml). The layers were separated and the aqueous phase was extracted repeatedly with ether. The ethereal extracts yielded a solid indistinguishable (n.m.r., t.l.c., g.l.c.) from the starting material. A sample (84 mg) recrystallised from methanol gave needles (39·6 mg.), m.p. and mixed m.p. with the ketone (12) 113—116°.

Treatment of the Ketone (12) *with Sodium Deuterioxide*.—The ketone (12) (100 mg) was treated as above but with sodium deuterioxide prepared from sodium (23 mg), deuterium oxide (99·7%; 4 ml), and dioxan (8 ml). The n.m.r. spectrum of the product displayed, *inter alia*, a singlet at τ 9·04 [C(4)Me]. A sample recrystallised from methanol had m.p. 114·5—117·5°.

The Dimesylate (13).—Methanesulphonyl chloride (50 ml) in pyridine (50 ml) was added in portions to a stirred solution of the ketone (5) (8·92 g) in pyridine (250 ml) at 0°. The solution was kept for 5 h at 0°, then added to ice–water (2 l). The mixture was adjusted to pH 2 with sulphuric acid and extracted with chloroform (2 × 1 l, 2 × 500 ml). The extracts were washed with N-sodium hydroxide (2 × 500 ml) and water (500 ml). Removal of the solvents gave 16-*oxo*-17-*noraphidicolan*-3α,18-*diyl bismethanesulphonate* (13) (12·8 g) as an oily solid. An analytical specimen, purified by chromatography on silica gel and recrystallised from ethanol, had m.p. 162—164° (Found: C, 54·6; H, 7·2. $C_{21}H_{34}O_7S_2$ requires C, 54·5; H, 7·4%), $\nu_{max.}$ 1715s, 1195m, 1180s, 1170s, 970s, and 900s cm.$^{-1}$.

Preparation of the Thioether (32).—A solution of the crude dimesylate (13), ethylene glycol (50 ml), and toluene-*p*-sulphonic acid (10 g), in benzene (600 ml) was boiled under reflux for 40 min in a Dean–Stark apparatus. The solution was cooled, added to chloroform (1 l) and washed repeatedly with N-sodium hydroxide. The solvents were evaporated and the residue, in dimethylformamide (125 ml), was added to a solution of sodium benzenethiolate prepared from benzenethiol (28·75 g), sodium hydride (0·5 g), and dimethylformamide (300 ml). The mixture was heated to 90° over 1 h and kept at 90° for 4 h. The mixture was cooled, added to water (2 l), and adjusted to pH 11 with 3N-sodium hydroxide. Extraction with ether afforded an oil which was treated with 1·5N-sulphuric acid (160 ml) in acetone (1 l) for 24 h at room temperature. Water (1 l) was added and the mixture was adjusted to pH 7·5. The bulk of the acetone was evaporated *in vacuo* and the resulting oily suspension set aside to crystallise. The product was separated by chromatography on silica gel (550 g). Benzene–ethyl acetate (9 : 1) eluted a solid (8·767 g) which was recrystallised from methanol to give 18-*phenylthio*-17-*noraphidicol*-2-*en*-16-*one* (32) (5·79 g) as plates, m.p. 129·5—133·5° (Found: C, 78·7; H, 8·4%; M^+, 380·2185. $C_{25}H_{32}OS$ requires C, 78·9; H, 8·5%; M, 380·2175), $\nu_{max.}$ ($CHCl_3$) 1710vs cm.$^{-1}$.

Desulphurisation of the Thioether (32).—A suspension of W-4 Raney nickel [21] (40 ml) in acetone (400 ml) was boiled under reflux for 2 h. The acetone was removed by decantation and the nickel was washed repeatedly with ethanol. A solution of the thioether (32) (1 g) in ethanol (100 ml) was added and the mixture was boiled under reflux for 18 min. Filtration of the mixture and evaporation of the solvent gave a solid (0·700 g). The products from five such reactions were combined and separated by chromatography on silica gel (135 g). Ethyl acetate–petroleum (1 : 40) eluted a fraction which was separated further by chromatography on 7% silver nitrate–silica gel (100 g). Benzene–petroleum (3 : 1) eluted 17-*noraphidicolan*-2-*en*-16-*one* (33) (1·71 g). A sample recrystallised from methanol gave needles, m.p. 112—115° (Found: C, 84·0; H, 10·2%; M^+, 272·2154. $C_{19}H_{28}O$ requires C, 83·8; H, 10·3%; M, 272·2140), $\nu_{max.}$ 1726s, 1096m, 1000m, 988m, and 720s cm.$^{-1}$.

Hydrogenation of the Enone (33).—The enone (33) (1·66 g) in ethanol (200 ml) containing Adams catalyst (300 mg) was hydrogenated for 1·5 h at room temperature and atmospheric pressure. The product was separated by chromatography on silica gel (250 g). Benzene–ethyl acetate (20 : 1) eluted a solid which was recrystallised from methanol to give 17-*noraphidicolan*-16-*one* (14) (0·771 g) as plates, m.p. 135—139° (Found: C, 83·1; H, 11·2%; M^+, 274·229. $C_{19}H_{30}O$ requires C, 83·2; H, 10·9%; M, 274·2296), $\nu_{max.}$ (CCl_4) 1723vs cm^{-1}.

The Olefins (15) *and* (34).—A stirred suspension of methyltriphenylphosphonium iodide (3·13 g) in tetrahydrofuran (50 ml) was treated for 1·5 h with 1·985M-butyl-lithium in hexane (2·86 ml). The ketone (14) (0·350 g.) in tetrahydrofuran (20 ml.) was added and the mixture was heated over 50 min to 70° and maintained at 70° for 2·5 h. The cooled solution was added to brine (500 ml) and extracted with ether (1 × 250, 3 × 150 ml). Removal of the solvents gave a residue which was separated by chromatography on silica gel (50 g). Petroleum eluted a solid which was separated further by p.l.c. on 15% silver nitrate–silica gel [solvent system toluene–petroleum (1 : 4)]. The material of lower R_F was recrystallised from methanol to give *aphidicol*-16-*ene* (15) (184·5 mg) as needles, m.p. 70—72·5°, $[\alpha]_D^{23·5}$ −13·2° (*c* 1·0, $CHCl_3$) (Found: C, 87·9; H, 11·6%; M^+, 272·2497. $C_{20}H_{32}$ requires C, 88·2; H, 11·8%; M, 272·2503), $\nu_{max.}$ 3080m, 1655m, and 885vs cm^{-1}. The material of higher R_F was recrystallised from methanol to give *aphidicol*-15-*ene* (34) (55·1 mg) as needles, m.p. 101—105°, $[\alpha]_D^{23·5}$ +11·2° (*c* 0·5, $CHCl_3$) (Found: C, 87·8; H, 12·0%; M^+, 272·2499. $C_{20}H_{32}$ requires C, 88·2; H, 11·8%; M, 272·2503), $\nu_{max.}$ 1210m, 1120m, 1010m, 1000m, 805m, and 785m cm.$^{-1}$.

The Acetonide (35).—A solution of the ketone (5) (4·2 g) and toluene-*p*-sulphonic acid (4·2 g) in acetone (250 ml) was boiled under reflux for 10 min, then cooled and poured into water (1 l). The mixture was adjusted to pH 7·5 and the bulk of the acetone was removed *in vacuo*. 3α,18-*Isopropylidenedioxy*-17-*noraphidicolan*-16-*one* (35) (4·24 g) crystallised directly from the aqueous mixture as needles, m.p. 141—144°. An analytical specimen recrystallised from ether–petroleum had m.p. 143—146° (Found: C, 76·3; H, 9·7. $C_{22}H_{34}O_3$ requires C, 76·3; H, 9·9%), $\nu_{max.}$ (CCl_4) 1724s, 1200m, and 1090m cm.$^{-1}$.

Reaction of the Ketone (35) *with Triphenylphosphonium Methylide*.—A stirred suspension of methyltriphenylphosphonium iodide (9·35 g.) in ether (200 ml.) was treated with

[21] A. A. Pavlic and H. Adkins, *J. Amer. Chem. Soc.*, 1946, **68**, 1471.

0·85M-ethereal phenyl-lithium (20 ml) for 2 h at 25°. The ketone (35) (1·32 g) in ether (100 ml) was added and the stirred mixture was left for 18 h at 25°. The ether was removed by distillation, the solvent volume being maintained constant by dropwise addition of tetrahydrofuran.[22] The mixture was boiled under reflux for 5·5 h, then cooled and added to water (250 ml). Repeated extraction with ether afforded an oily solid which was triturated several times with petroleum. The petroleum-soluble fraction was separated by chromatography on silica gel (100 g). Petroleum–benzene (1 : 3) eluted 3α,18-*isopropylidenedioxyaphidicol*-16-*ene* (36) (0·97 g) as needles, m.p. 120—134°. A specimen recrystallised twice from methanol had m.p. 134—135° (Found: C, 80·5; H, 10·2. $C_{23}H_{36}O_2$ requires C, 80·2; H, 10·5%), $\nu_{max.}$ 3075m, 1653m, 1095vs, and 879vs cm^{-1}.

The Olefin (16).—A mixture of the olefin acetonide (36) (800 mg), toluene-*p*-sulphonic acid (417 mg), methanol (420 ml), and water (17 ml), was stirred for 23 h at room temperature, then added to ether (1 l). The ethereal layer was washed with saturated sodium hydrogen carbonate (3 × 100 ml) and water (3 × 100 ml). Evaporation of the solvents afforded a solid (650 mg) which was separated by chromatography on silica gel (70 g). Ethyl acetate–benzene (3 : 2) eluted a solid (584 mg) which was recrystallised from ethyl acetate–cyclohexane to give *aphidicol*-16-*ene*-3α,18-*diol* (16) (356 mg) as needles, m.p. 147—148° (Found: C, 78·6; H, 10·6%. $C_{20}H_{32}O_2$ requires C, 78·9; H, 10·6%), $\nu_{max.}$ 3250s, 1653m, 1213m, 1043s, 882s, and 760s cm^{-1}.

Reduction of the Keto-diacetate (6) *with Lithium Aluminium Hydride*.—A mixture of the keto-diacetate (6) (689 mg), lithium aluminium hydride (350 mg), and tetrahydrofuran (60 ml) was stirred for 30 min at room temperature. The excess of reducing agent was destroyed by dropwise addition of ethyl acetate and the mixture was added to water (750 ml), acidified, and extracted with ethyl acetate. A solution of the product and toluene-*p*-sulphonic acid (200 mg) in acetone (125 ml) was boiled under reflux for 20 min. The mixture was cooled, adjusted to pH 9, and extracted with ether to give a solid (631 mg), a sample of which (290 mg) was purified by p.l.c. using benzene–ethyl acetate (9 : 1). The major component was recrystallised from methanol to give 3β,18;16,17-*bisisopropylidenedioxyaphidicolane* (41) (67 mg) as needles, m.p. 215—218° (Found: C, 74·5; H, 10·2. $C_{26}H_{42}O_4$ requires C, 74·6; H, 10·1%), $\nu_{max.}$ 1254s, 1210s, 1080s, 1065s, and 870s cm^{-1}.

Hydrolysis of the Bisacetonide (41).—A solution of the bisacetonide (41) (312 mg), toluene-*p*-sulphonic acid (100 mg) and water (10 ml) in methanol (150 ml), was left for 10 days at room temperature, then added to water (350 ml). The mixture was adjusted to pH 8 and the bulk of the methanol was evaporated *in vacuo*. Extraction of the residue with ethyl acetate gave a sticky solid (239 mg) which was recrystallised from ethyl acetate. Two further recrystallisations of the product from aqueous methanol gave 3-epi-*aphidicolin* (*aphidicolane*-3β,16,17,18-*tetraol*)(17) as needles, m.p. 173—175·5° (Found: C, 71·1; H, 9·8. $C_{20}H_{34}O_4$ requires C, 71·0; H, 10·1%), $\nu_{max.}$ 1065s, 1045s, 1025s, 1018 s, and 970m cm^{-1}.

Acetylation of the Tetraol (17).—Acetylation of the tetraol (17) with acetic anhydride in pyridine gave a triacetate as an oil which could not be crystallised [M^+, 391·2486. ($C_{26}H_{40}O_7$ — CH_2OAc) requires 391·2484], τ ($CDCl_3$) 5·3-[m, $W_{\frac{1}{2}}$ 16·75 Hz C(3)H], 6·06(s) and 6·29(s) (CH_2OAc), 7·94(s), 7·97(s), and 8·02(s) (OAc) and 9·01(s) and 9·17(s) CMe).

Periodate Cleavage of the Tetraol (17).—The tetraol (17) (66·5 mg) in acetic acid (3 ml) and water (1·5 ml) was treated with periodic acid (5% w/w; 0·25 ml) for 20 min at room temperature, then poured into water (25 ml). The solution was made alkaline and extracted with ethyl acetate (3 × 25 ml). Evaporation of the solvent and recrystallisation of the residue from ethyl acetate–petroleum gave 3β,18-*dihydroxy*-17-*noraphidicolan*-16-*one* (18) (48 mg) as needles, m.p. 196—198·5°. An analytical specimen had m.p. 199—202° (Found: C, 74·2; H, 9·9. $C_{19}H_{30}O_3$ requires C, 74·5; H, 9·9%), $\nu_{max.}$ (CCl_4) 1725s cm^{-1}.

Reaction of the Acetonide (35) *with Dimethylsulphoxonium Methylide*.—(a) The acetonide (35) (3·18 g), in tetrahydrofuran (25 ml), was treated with the ylide prepared[23] from trimethylsulphoxonium iodide (8·15 g), sodium hydride (60%; 1·41 g), and dimethyl sulphoxide (120 ml) for 2 h at room temperature and for a further 45 min at 50°. The mixture was cooled, poured into water (1·5 l), and extracted with ether (4 × 400 ml) to yield a mixture of the epoxides (42) and (43). Three recrystallisations of the product from methanol afforded 16,17-*epoxy*-3α,18-*isopropylidenedioxyaphidicolane* (42) (0·671 g), m.p. 158·5—161° (Found: C, 76·6; H, 10·0%; M^+, 360. $C_{23}H_{36}O_3$ requires C, 76·7; H, 10·0%; *M*, 360), $\nu_{max.}$ 1200s, 1090s, 1005s, and 910s cm^{-1}.

(b) A solution of the crude epoxide mixture (506 mg) prepared by the foregoing procedure, and potassium hydroxide, in dioxan (50 ml) and water (87 ml), was maintained at 100° for 16 h. The solution was cooled, diluted with water (1 l) and extracted with ether (4 × 250 ml). Evaporation of the solvents gave an oil which was treated with toluene-*p*-sulphonic acid (600 mg) and acetone (50 ml) for 25 min at reflux temperature. The cooled solution was diluted with water (250 ml), adjusted to pH 9 with sodium carbonate, and extracted with ether (400 ml) to yield a mixture of epimeric bisacetonides which were separated by p.l.c., each plate being developed twice with ethyl acetate–carbon tetrachloride (1 : 19). The material (247 mg) of R_F 0·44 was recrystallised twice from methanol to give the bisacetonide (20) (129 mg), m.p. 145—146°, identified by comparison with an authentic specimen. The material (165·5 mg) of R_F 0·25 was recrystallised three times from methanol to give 3α,18;16,17-*bisisopropylidenedioxy*-16-epi-*aphidicolane* (44) (55 mg) as needles, m.p. 175—178° (Found: C, 75·0; H, 9·9. $C_{26}H_{42}O_4$ requires C, 74·6; H, 10·1%), $\nu_{max.}$ 1263s, 1238s, 1195s, 1092s, 1058s, and 870s cm[81].

Hydrolysis of the Epoxide (42).—A solution of the epoxide (42) (50 mg) and sodium hydroxide (500 mg) in dioxan (5 ml) and water (7·7 ml) was maintained at 100° for 16 h, then cooled and diluted with water (100 ml). Extraction with ether afforded an oil which slowly solidified. Treatment of the solid with toluene-*p*-sulphonic acid in refluxing acetone gave a single product inseparable from aphidicolin bisacetonide (20) by analytical t.l.c. in ethyl acetate–carbon tetrachloride (1 : 19).

Hydrolysis of the Bisacetonide (44).—A stirred solution of the bisacetonide (44) (100 mg) and toluene-*p*-sulphonic acid (80 mg) in water (8 ml) and methanol (80 ml) was left for 6 days at room temperature, then added to water (250

[22] F. Sondheimer and R. Mechoulam, *J. Amer. Chem. Soc.*, 1957, **79**, 5029.

[23] J. D. Ballantine and P. J. Sykes, *J. Chem. Soc.* (*C*), 1970, 731.

ml). The mixture was made alkaline with 3N-sodium hydroxide and the bulk of the ethanol was evaporated off *in vacuo*. Extraction of the resulting suspension with ethyl acetate (4 × 100 ml) afforded 16-epi-*aphidicolin* (19) as an amorphous solid, m.p. 128—130°, which could not be crystallised (Found: C, 70·6; H, 10·1. $C_{20}H_{34}O_4$ requires C, 71·0; H, 10·1%), $\nu_{max.}$ 1078s, 1043s, 1028s, and 970 cm^{-1}.

Periodate Cleavage of the Tetraol (19).—A solution of the tetraol(19) (47 mg) in acetic acid (2 ml) and water (0·5 ml) was treated with periodic acid (50% w/w; 0·2 ml) for 10 min at room temperature. The solution was diluted with water, adjusted to pH 9, and extracted with chloroform (4 × 25 ml). Removal of the solvent gave a solid which was purified by p.l.c. in benzene–ethyl acetate–methanol (50 : 50 : 1). Recrystallisation of the product from ethyl acetate–light petroleum gave 3α,18-*dihydroxy*-17-*noraphidicolan*-16-*one* (5) (24 mg), m.p. and mixed m.p. with an authentic sample 154·5—156°.

Reaction of the Acetonide (35) *with Dimethylsulphonium Methylide*.—The acetonide (35) (0·885 g) in tetrahydrofuran (5 ml) and dimethylsulphoxide (5 ml.) was treated with the ylide prepared [23] from trimethylsulphonium iodide (2·115 g), sodium hydride (60%; 400 mg.) and dimethyl sulphoxide (30 ml) for 15 min at 0° and for a further 30 min at room temperature. The mixture was added to water (75 ml) and extracted with ether to afford the mixture of epoxides (42) and (43) as a gummy solid.

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