Metabolites of the Higher Fungi. Part 24.[1] Cytochalasin N, O, P, Q, and R. New Cytochalasins from the Fungus *Hypoxylon terricola* Mill

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Punctaporonin B, cytochalasin C and D, and five new cytochalasins have been isolated from the culture medium and mycelium of the fungus *Hypoxylon terricola*. Cytochalasin C is the major metabolite. Cytochalasin N is the 5,6-epoxide of cytochalasin C, and cytochalasins O and P are two epimeric 6-hydroxy analogues. Cytochalasin Q is a 6,7-epoxide and R is a 6,7,13,14-diepoxide. The epoxides formed by cytochalasin C and D are described and the acid- and BF_3-induced rearrangement products of cytochalasin N are identified.

The cytochalasins are a group of toxic fungal metabolites which show marked cytostatic effects on mammalian cells in tissue culture. They were first isolated and characterised by Aldrich and Turner[2] at Imperial Chemical Industries and independently by Tamm[3] in Zurich, and since then they have been identified as metabolites of a number of fungi. Reports of such compounds originating from xylariaceous fungi are confined to two species. Cytochalasin E (**1**) is a metabolite of *Rosellinia necatrix*[4,5] and engleromycin (**2**) is produced by *Engleromyces goetzei*;[6,7] cytochalasin E is unique in possessing a carbonate ester function and engleromycin is the 19,20-epoxide of deacetyl cytochalasin D and is the only compound to date found to bear an epoxy function in the macrocyclic ring.

In the previous paper[1] we described the isolation and characterisation of the punctaporonins, a group of new sesquiterpenoid compounds from the xylariaceous dung fungus *Poronia punctata*, and it was the occurrence of these compounds which prompted us to consider their possible formation by members of the genus *Hypoxylon* and especially by the members of the sub-section primocinerea, since members of this sub-section appear to show affinity with species of other xylariaceous genera. *Hypoxylon terricola* is an uncommon member of the sub-family with a habitat restricted to dead and decaying coniferous needles; in this habitat it shows affinity to *P. punctata*. The species is only found at one location in the U.S.A. and has recently been found in the Atlantic Pyrenees.

Solvent extraction of the malt extract growth medium gave a gummy solid comprising a mixture of punctaporonin B (**3**), cytochalasin C (**4**) and D (**5**), and the new cytochalasins N (**6**), O (**7**), P (**8**), Q (**9**), and R (**10**). Extraction of the mycelium produced a mixture of C, D, N, and O. On t.l.c. silica plates the new compounds give a red (N), yellow (O, P, Q), or blue (R) colouration after spraying with an anisaldehyde–acetic acid–sulphuric acid (1:98:1) mixture followed by heating at 110 °C for 2—3 min.

Cytochalasin N (**6**), $C_{30}H_{37}NO_7$, *m/z* 523 contains one more oxygen atom than cytochalasin C or D. With dilute alkali the compound is hydrolysed with loss of AcO and is monoacetylated with acetic anhydride–pyridine. These properties parallel those of C and D and the co-occurrence of these two compounds suggested a common basic skeleton. The relationship with C was confirmed in the ^1H n.m.r. spectrum (Table) by the presence of four methyl signals in the range δ 1—2 comprising three singlets and a doublet, and an acetate methyl at δ 2.38; *trans* C-19,20 and C-13,14 olefinic protons also occur in positions similar to those in cytochalasin C. The main differences are in the reduced isoindolone nucleus; 7-H is upfield (Δδ 0.31) and 3-H and 8-H downfield (Δδ 0.36 and 0.72 respectively). The two benzylic protons appear as a doublet (δ 3.16) compared with the two separate doublet of doublets at δ 3.15 and 3.22 in cytochalasin C. In the ^{13}C n.m.r. spectrum only ten aromatic and olefinic carbons occur above δ 120 compared with twelve in C; the missing carbons are quaternary and now appear at δ 63.4 and 65.7. This data is compatible with the presence of an epoxide function at the 5,6 position. Acid hydrolysis of (**6**) yielded a 5,6-dihydroxy derivative (**11**) of C confirming the presence of the epoxide ring. Additional proof of the presence of the latter in the cyclohexane ring was obtained by alkaline hydrolysis[8] of (**6**) to yield the unsaturated epoxy acid (**12**) which was hydrolysed by acid to the trihydroxy acid (**13**).

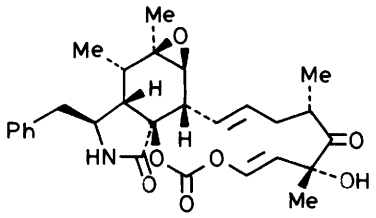
The occurrence of an epoxide function at the 5,6-positions in naturally occurring cytochalasins is unique. The known epoxides, *e.g.* cytochalasins E,[5,9] F,[5,9] G,[10,11] epoxy H,[12] K,[13] L,[13] and M[13] and the chaetoglobosins A,[14] C,[14,15] F,[16] and K[17,18] are all 6,7-epoxides. The action of aqueous acid on cytochalasins E and F does not yield a glycol.[9] Instead, both undergo isomerisation to yield the allylic 5,6- or 6,12-en-7-ol. Similar rearrangements are reported for chaetoglobosin A with triethylamine–BF_3 or when kept in chloroform solution;[14] F behaves similarly in boiling acetic acid.[16] In an attempt to induce this type of rearrangement in the 5,6-epoxide (**6**), a suspension in acetonitrile was treated with BF_3·OEt_2. Solution occurred slowly with the formation of a mixture of an aldehyde and a ketone (**6a**) and (**6b**), both isomeric with the starting material.

The aldehyde (**6a**) was identified by a one proton singlet at δ 9.4 and a methine carbon at δ 203.99 in $CDCl_3$ (10.06 and 205.17 in [$^2H_5$]pyridine). The compound retains the characteristic ^1H n.m.r. resonances of the macrocyclic ring and its formation can be explained (Scheme) by a pinacol–pinacolone type rearrangement[19] of the BF_3–epoxide complex. Such a rearrangement is permissible where the migrating C–C bond is *trans*-anticoplanar to the epoxide C–O bond. The structure is supported by the appearance of the 10- and the 11-methyl signals as singlets and 7-H is a doublet coupled only to 13-H. Ring closure of (**6d**) is indicated by the presence of only ten aromatic and olefinic carbons in the range δ 120—140 in the $CDCl_3$ ^{13}C n.m.r. spectrum (in [$^2H_5$]pyridine only eight are observed). In addition, there are two quaternary carbons linked

Table. ^{1}H N.m.r. chemical shifts (δ, relative to Me_4Si in C_5D_5N)

	N (6)[a]	O (7)[a]	P (8)[a]	Q (9)[b]	R (10)[b]
2-NH	9.55 (s)	9.25 (s)	9.10 (s)	9.5 (s)	9.7 (s)
3-H	4.05 (ddd, 7.0, 7.0, 2.0)*	3.98 (ddd, 7.6, 5.6, 4.6)*	4.79 (ddd, 8.0, 4.5, 4.0)*	3.92 (dd, 6.6, 7.9)**	3.96 (ddd, 7.0, 7.1, 2.0)**
4-H	2.88 (d, 2.0)	2.43 (dd, 5.3, 4.6)	2.30—2.41 (m, 2 H)	2.45 (dd, 5.7, 1.8)	2.40 (dd, 5.7, 2.0)
5-H	—	2.6—2.8 (m, 3 H)	2.30—2.41 (m, 2 H)	1.90—2.05 (dq, 7.3, 5.7, 2 H)	1.85 (dq, 5.7, 7.3)
6-H	—	—	—	—	—
7-H	4.15 (dd, 10.5, 6.0)	3.76 (d, 11.5)	4.29 (dd, 11.0, 3.0)	3.06 (d, 5.8, 2 H)	3.39 (d, 5.7)
8-H	3.81 (dd, 10.5, 10.5)	3.55 (dd, 10.0, 11.5)	3.26 (dd, 11.0, 10.3)	2.97 (dd, 9.9, 5.7)	1.98 (dd, 8.6, 5.7, 2 H)
10-H_2 H_a	3.16 (d, 7.0)	2.95 (dd, 13.5, 5.2)	2.60—2.85 (m, 3 H)	2.84 (dd, 13.2, 7.9)	2.84 (dd, 13.2, 7.7)
10-H_2 H_b		2.84 (dd, 13.5, 7.6)	3.12 (dd, 13.7, 3.4)	3.09 (dd, 13.2, 6.6, 2 H)	3.11 (dd, 13.2, 6.4, 2 H)
11-H_3	1.59 (s)	1.14 (d, 6.3)	1.39 (d, 6.9)	0.74 (d, 7.3)	0.71 (d, 7.3)
12-H_3	1.25 (s)	1.44 (s)	1.72 (s)	1.21 (s)	1.20 (s)
13-H	6.61 (dd, 15.5, 10.5)	6.32 (dd, 15.3, 10.0)	6.33 (dd, 15.0, 9.8)	6.46 (dd, 15.6, 10.1)	4.37 (dd, 8.6, 2.0)
14-H	5.47 (ddd. 15.5, 10.5, 4.5)	5.43 (ddd, 15.3, 10.7, 4.7)	5.47 (ddd, 15.0, 10.6, 4.4)	5.46 (ddd, 15.6, 10.4, 5.2)	3.0—3.12 (m, 3 H)
15-H_2 H_α	1.95 (ddd, 11.5, 5.5, 1.0)	1.97 (dd, 12.3, 4.4)	2.01 (dd, 12.5, 4.4)	1.90—2.05 (m, 2 H)	1.95—2.09 (m, 2 H)
15-H_2 H_β	2.58—2.75 (m, 2 H)	2.6—2.8 (m, 3 H)	2.60—2.85 (m, 3 H)	2.64—2.77 (m, 2 H)	2.12 (dd, 12.4, 1.5)
16-H	2.58—2.75 (m, 2 H)	2.6—2.8 (m, 3 H)	2.60—2.85 (m, 3 H)	2.64—2.77 (m, 2 H)	3.0—3.12 (m, 3 H)
19-H	5.65 (dd, 16.0, 2.5)	5.64 (dd, 15.9, 2.5)	5.67 (dd, 15.9, 2.4)	5.62 (dd, 15.8, 2.4)	6.22 (dd, 12.5, 2.6)
20-H	6.76 (dd, 16.0, 2.5)	6.98 (dd, 15.9, 2.5)	7.06 (dd, 15.9, 2.8)	6.85 (dd, 15.8, 2.4)	7.18 (dd, 12.5, 2.6)
21-H	6.22 (dd, 2.5, 2.5)	5.94 (dd, 2.5, 2.5)	5.94 (dd, 2.6, 2.6)	6.24 (dd, 2.2, 2.4)	6.25 (s)
22-H_3	1.02 (d, 6.5)	1.04 (d, 6.6)	1.06 (d, 6.8)	1.07 (d, 6.2)	1.03 (d, 6.8)
23-H_3	1.56 (s)	1.56 (s)	1.60 (s)	1.57 (s)	1.62 (s)
21-OAc	2.38 (s)	2.31 (s)	2.43 (s)	2.38 (s)	2.30 (s)
6-OH	—	5.37 (s, br)[c]	4.9[e]	—	—
7-OH	6.06 (d, 6.0)	6.08 (s, br)[d]	5.80 (d, 3.0)	—	—
18-OH	6.02 (s, br)	5.99 (s, br)[c]	6.01 (s)	6.05—6.15 (s, br)	6.54 (s)
Ar-5H	7.29—7.38 (m)	7.22—7.34 (m)	7.16—7.32 (m)	7.2—7.4 (m)	7.20—7.38 (m)

Operating frequency: [a] 400 MHz, [b] 270 MHz; H overlapping signals; * broadened; ** slightly broadened. [c] Assignments may be reversed. [d] These signals can also couple and appear as a dd and d respectively (*J* 6.6 Hz) in dry C_5D_5N. [e] Under HOD signal; in a 270 MHz spectrum appeared at δ 6.28; position appears to vary.



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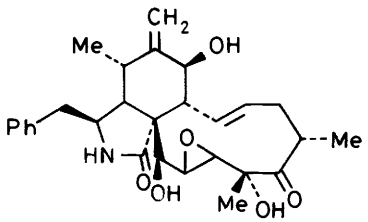

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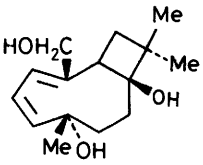

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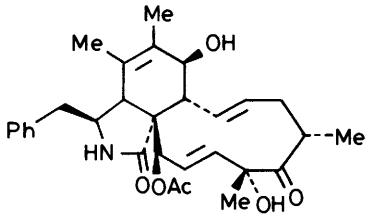

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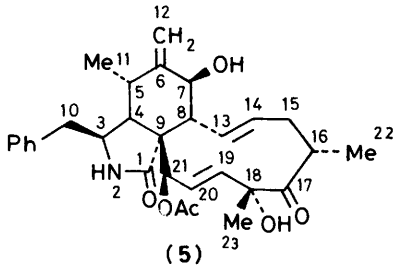

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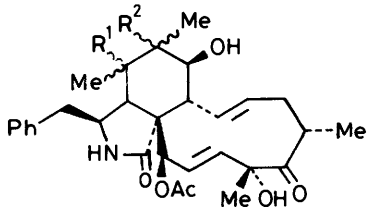
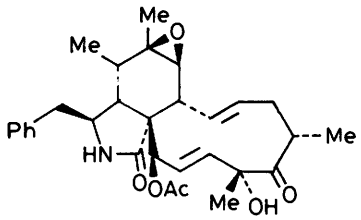


(6) and (18) R^1, R^2 = O

(7) and (8) R^1 = H, R^2 = OH

(11) R^1 = R^2 = OH



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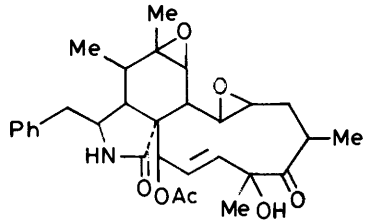

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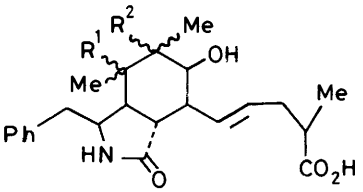
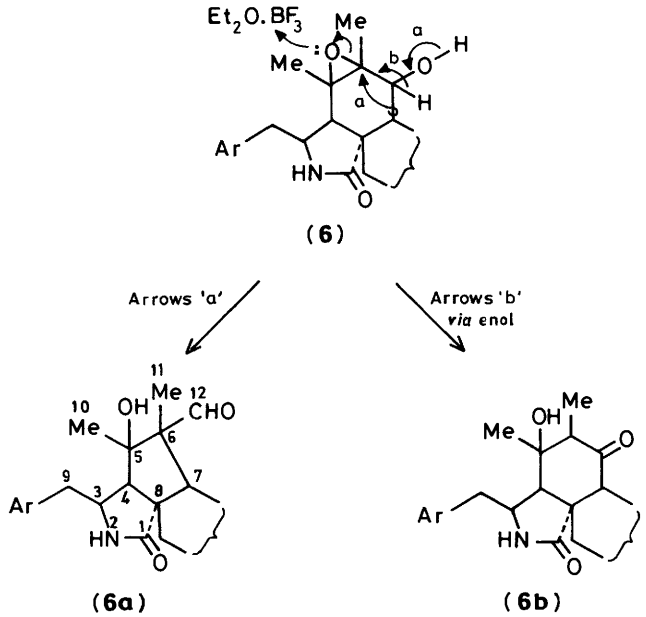


(12) R^1 = R^2 = O

(13) R^1 = R^2 = OH

(14) R^1 = H, R^2 = OH



(6)

(6a)

(6b)

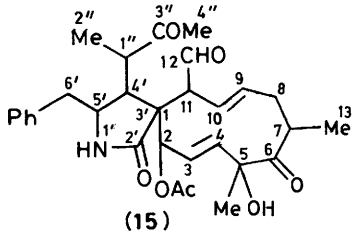
Scheme.

to oxygen at δ 80.75 and 77.95 (C-5 and C-18) and two others at δ 59.86 and 64.60 (C-6 and C-8) not linked to oxygen.

The ketone (**6b**) was identified by the presence of an additional quaternary carbon at δ 210.95 and its formation can be explained (Scheme) by the rearrangement to the enol which isomerises. This structure is confirmed by the appearance of the 11- and 12-methyl signals as a singlet and a doublet respectively.

Cytochalasin O (**7**), $C_{30}H_{39}NO_7$, *m/z* 525, is slightly more polar than N in the solvent system used. Its close relationship with C and N was established by the formation of a mono-acetate and the loss of acetate on hydrolysis. In the ^1H n.m.r. spectrum the acetate methyl and the 13,14- and 19,20-olefinic protons occur in their expected positions. However, the other four methyls now appear as two doublets and two singlets, and since the molecular formula differs from that of N by only two hydrogen atoms, this implies that the compound is an alcohol, presumably derived from the epoxide (N) by biological reduction. The relative locations of the proton and the OH group were obtained from both chemical and spectroscopic evidence. Alkaline hydrolysis yielded the unsaturated acid (**14**) proving that the additional oxygen is located in the cyclohexane ring, and periodate oxidation gave the keto-aldehyde (**15**). The ^{13}C n.m.r. spectrum of O, like that of N, shows ten aromatic and olefinic carbons above δ 120. However, unlike N, C-5 appears as a non-oxygenated methine absorption at δ 39.85 and C-6 appears as an oxygenated quarternary at δ 72.56. In the ^1H n.m.r. spectrum 4-H is coupled to 3-H and 5-H and appears as a doublet of doublets (*J* 4.6 and 5.3 Hz). In this compound the benzylic protons occur as two separate doublets of doublets like those in C, but unlike those in N.

Cytochalasin P (**8**), $C_{30}H_{39}NO_7$, *m/z* 525, is isomeric with O (**7**) and can be separated from the latter by virtue of its much



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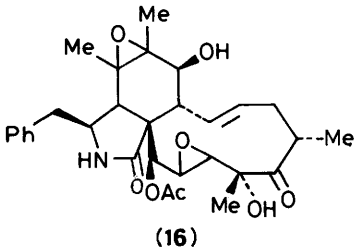

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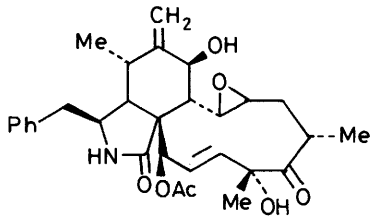

(17)

greater solubility in most organic solvents. The ^{13}C n.m.r. is not significantly different from that of (**7**) and in the ^{1}H n.m.r. spectrum all the protons characteristic of (**7**) are identifiable. However, there are marked shift differences among those protons associated with the reduced isoindolone ring; 3-H and 7-H are both significantly downfield (3-H $\Delta\delta$ 0.81, 7-H $\Delta\delta$ 0.53) and 8-H is upfield ($\Delta\delta$ 0.29), also 4-H and 5-H occur close together near δ 2.35 and 5-H is at higher field ($\Delta\delta$ 0.35) than in (**7**). Evidence that the hydroxy group resides on C-6, thus making this compound the 6-epimer of (**7**), is obtained from similar couplings of 8-H to 7-H and 5-H to 4-H as occurs in (**7**).

Cytochalasin Q (**9**) and R (**10**) are the least polar metabolites in the chromatographic system used and were the most difficult to purify. They exist as glassy solids when free of solvent; Q yields fine solvated needles from acetone and R was obtained as large solvated rhombs from ethyl acetate or solvated needles from methyl cyanide.

Cytochalasin Q (**9**) $C_{30}H_{37}NO_6$, *m/z* 507 is isomeric with C but is much more soluble in organic solvents. In its ^{1}H n.m.r. spectrum there is an acetate methyl and four other methyl signals, but unlike C two of these are doublets and in [$^{2}H_5$]-pyridine the 11-methyl is at abnormally high field (δ 0.75). The 13,14- and 19,20-olefinic protons occur in their normal downfield positions but 7-H is now at a much higher field (δ 3.06). The isomeric nature of this compound with C and the occurrence of the 12-Me as a singlet and the 11-Me as a doublet suggested an epoxide linkage across the 6,7-positions. This is supported in the ^{13}C n.m.r. by the occurrence of ten methine and quaternary unsaturated carbons in the range δ 120—140, two oxygenated quarternaries at 78.3 and 57.3 (C-18 and C-6), and two oxygenated methine carbons at 76.6 and 63.2 (C-21 and C-7). Chemical proof was obtained by the almost instantaneous isomerisation to cytochalasin C at room temperature in the presence of a trace of mineral acid; in this property it shows a close analogy with other cytochalasin 6,7-epoxides. Cytochalasin D was not detected as a product of this isomerisation.

Cytochalasin R (**10**) $C_{30}H_{37}NO_7$, *m/z* 523 is isomeric with N. In the ^{1}H n.m.r. spectrum ([$^{2}H_5$]pyridine) there is an acetate methyl and four others, two of which are doublets and as in Q the 11-Me is at high field (δ 0.75). The 19,20-olefinic protons occur in their expected low-field positions. However, the 13,14-protons occur at δ 4.37 and 3.0—3.12 respectively; *i.e.* at much higher field than in any known cytochalasin and their coupling constant is only 2 Hz. In addition 8-H is displaced upfield from δ 2.97 (in Q) to δ 1.98, suggesting epoxidation at C-13,14; the low *J* value is typical of a *trans* epoxide. Because the 11- and 12-methyl groups are a doublet and a singlet respectively, epoxidation across C-6 and C-7 is also indicated. Additional support for the diepoxide structure is obtained from the ^{13}C n.m.r. spectrum which compared with that of N shows only eight methine and quaternary unsaturated carbons in the range δ 120—140 and two additional methine carbons at δ 50—65. Chemical proof of the location of the epoxide across the 6,7-positions was obtained by treating R with a trace of acid; this resulted in a mixture whose ^{1}H n.m.r. spectrum showed the disappearance of the high-field doublet indicative of isomerisation at C-5 as in Q. At least three compounds appear to be formed in this reaction, but the mixture could not be separated satisfactorily.

The occurrence of the 5,6- and 6,7-epoxides as co-metabolites of the same fungus poses interesting questions. Is cytochalasin Q the precursor of C and is N formed from C? N is presumably the origin of O and P but there appears to be no similar reduction product of Q though there is considerable gummy material of still undetermined constitution.

In order to establish the ease and site of epoxidation, cytochalasin C was treated with *m*-chloroperbenzoic acid. Three products were formed. The major product and least polar on SiO_2 in the solvent system used was identical with cytochalasin N (**6**). The most polar product, $C_{30}H_{37}NO_8$, *m/z* 539 was identified as a diepoxide whose ^{13}C n.m.r. spectrum shows four unsaturated carbons less than cytochalasin C; these now appear as two oxygenated quaternary carbons at δ 63.56 and 65.85 indicative of 5,6-epoxide formation, and two oxygenated methine carbons at δ 54.13 and 57.18. The 5,6;19,20-diepoxy structure (**16**) was elucidated from the ^{1}H n.m.r. spectrum; the 19,20-proton absorptions are displaced to higher field and the 13,14-protons are unaffected.

The product formed in lowest yield and of intermediate polarity is also a mono-epoxide and was identified as isocytochalasin N (**18**); the 5,6-stereoisomer of N. In the ^{1}H n.m.r. spectrum (**18**) shows the 19,20- and the 13,14-double bond protons in their expected, but different positions from those of (**6**). In addition, there are major differences in some shift positions; 8-H is upfield ($\Delta\delta$ 0.75) and the 12-Me appears at δ 1.00 compared with δ1.25 in N. The ^{13}C spectrum is similar to that of (**6**) except that one of the 5,6-quaternary carbons is displaced down field ($\Delta\delta$ 3.91). The compound is hydrolysed by dilute aqueous acid to the same diol as is produced from (**6**).

Cytochalasin D (**5**) was similarly treated with *m*-chloroperbenzoic acid to yield a mono-oxygenated product. This was identified as the 13,14-epoxide (**17**) since in the ^{1}H n.m.r. spectrum the 19,20-olefinic protons and the two exocyclic unsaturated methylene protons occur as in the parent;[20] however the 13,14-protons are displaced upfield and occur in a similar position to those found in cytochalasin R (**10**). In addition, the 11-Me is now at high-field (δ 0.74 in [$^{2}H_5$]pyridine); this is a position similar to that found in R and further confirms the structure assigned to this latter compound.

Epoxidation of double bonds is a common biosynthetic reaction and leads to the formation of phenols and alcohols; epoxide formation is also the initiating reaction leading to many cyclisations as exemplified by the role of squalene oxide in terpene biosynthesis. The occurrence of a 6,7-epoxide function is common amongst naturally occurring cytochalasins and the facile rearrangement of 6,7-epoxides under acid conditions to allylic 5,6- and 6,12-en-7-ol systems suggests that cytochalasins such as C and D may be formed *in vivo* from 6,7-epoxide

precursors. Substance is added to this argument since cytochalasin N, a 5,6-epoxide, does not form an allylic alcohol system on treatment with acid, but gives a fully substituted 5,6-diol. The concurrent isolation of 5,6-epoxide, 6,7-epoxide, and 6,7;13,14-diepoxide systems from *Hypoxylon terricola* suggests that it possesses (i) 6,7-epoxide isomerase enzymes which produce cytochalasins C and D, possibly *via* cytochalasin Q, (ii) a 5,6-epoxide reductase enzyme which yields cytochalasin O and P possibly from N. The presence of cytochalasin R suggests that the enzymes responsible for opening the 5,6- and 6,7-epoxides are selective in their mode of action, since in R the 6,7;13,14-diepoxide system remains intact.

Epoxidation at 13,14- suggests that other compounds with the macrocyclic portion of the molecule modified at these positions may be found in the future.

Experimental

M.p.s were determined on a Kofler hot-stage apparatus, i.r. spectra on a Perkin-Elmer 681 spectrophotometer, u.v. spectra on a Unicam S.P. 800 spectrophotometer, ^1H and ^{13}C n.m.r. spectra on a Jeol-JNM GX-270, or Bruker WM-400 spectrometer (with $SiMe_4$ as an internal standard), mass spectra on an AEI 902 spectrometer, and optical rotations on a Perkin-Elmer 141 polarimeter. All ^1H and ^{13}C n.m.r. spectra are at 400 MHz and 100 MHz respectively for [$^2H_5$]pyridine solutions unless otherwise stated. However, connectivities in the ^1H n.m.r. spectra were established at 270 MHz using a combination of spin–spin decoupling, COSY and double quantum filter experiments. Carbon atom types were established in the ^{13}C n.m.r. using a combination of broad-band proton decoupling and DEPT experiments. All t.l.c., preparative t.l.c. (p.l.c.), and column chromatography was done on Merck Kieselgel PF 256 + 366 and flash chromatography on Merck Kieselgel 60 (230—400 mesh ASTM). Components on t.l.c. plates were identified by the colours produced when sprayed with AcOH–H_2SO_4–anisaldehyde (98:1:1) mixture and heated at 100—110 °C for 2—3 min. Extracts were dried over Na_2SO_4. Solvated compounds were desolvated for ^1H and ^{13}C n.m.r. by dissolution in pyridine and removal of the pyridine at 60 °C under reduced pressure.

Isolation of Cytochalasin C (**4**), *D* (**5**), *N* (**6**), *O* (**7**), *P* (**8**), *Q* (**9**), *R* (**10**), *and Puntaporonin B* (**3**) *from* Hypoxylon terricola.—The fungus was grown for 8 weeks in Thompson bottles (1 l); each contained malt extract (2% Edme; 500 ml). A thin yellow mycelium was removed by filtration through muslin and air died.

Filtrate. The filtrate (50 l) was extracted in batches (5 l) with ethyl acetate (3 × 1 l). The dried solvent was evaporated and the residual gummy solid (21.9 g) triturated with ethyl acetate (250 ml). The mixture was set aside overnight and filtered to yield a solid (5.4 g) which comprised a mixture of cytochalasins C, D, N, and O. The filtrate was evaporated and the residual gum from two such extractions (28.2 g) applied to a column of SiO_2 (4 × 50 cm) in chloroform–methanol (95:5) and eluted with the same solvent; 5 ml fractions were collected and evaporated.

Tubes 1—9. These gave a gum (0.33 g) which was discarded.

Tubes 10—39. These gave a viscous yellow gum (11.0 g) which was dissolved in ethyl acetate (50 ml). After 24 h the mixture was filtered to yield additional mixed *cytochalasin C, D, N,* and *O* (1.4 g). The mother liquor (m.l.) was evaporated and the gum redissolved in ethyl acetate (10 ml). Filtration after 24 h gave large rhombs (3.7 g) comprising mainly cytochalasin R. The viscous filtrate containing the bulk of the cytochalasin Q was evaporated and the resulting gum (5.7 g) dissolved in a mixture of benzene–ethyl acetate–acetic acid (50:49:1) and applied to a column of silica gel. Elution with the same solvent mixture and evaporation gave two fractions. (a) A gummy foam (yellow spot) yielding fine, silky, acetone solvated needles (0.38 g) of (7S,16S,18R,21R;13E,19E)-21-*acetoxy*-6,7-*epoxy*-18-*hydroxy*-16,18-*dimethyl*-10-*phenyl*[11]*cytochalasa*-13,19-*diene*-1,17-*dione* (*cytochalasin Q*) (**9**), m.p. 145—147 °C from acetone–light petroleum (b.p. 80—100 °C) [Found: C, 70.1; H, 7.3; N, 2.7. $C_{30}H_{37}NO_6 \cdot (CH_3)_2CO$ requires C, 70.1; H, 7.7; N, 2.5%]; *m/z* 507; $[\alpha]_D^{23}$ (solvate) −94.5° (*c* 1.0 in $CHCl_3$); $\nu_{max.}$(KBr) 3 440, 1 745, and 1 702 cm^{-1}; δ_C 12.58, 19.39, 19.63, 20.58, and 24.69 (CH_3), 38.30 and 46.19 (CH_2), 37.36, 42.49, 46.03, 50.54, 54.49, 63.16, 76.64, 127.05, 128.60, 129.00 × 2, 130.10 × 2, 131.94, 132.14, and 133.03 (CH), and 56.11, 78.26, 138.26, 170.66, 175.47, and 210.88 (C).

(b) A gum (bright blue spot) yielding rhombs (90 mg) of impure cytochalasin R from ethyl acetate. These were combined with the main bulk of the crude cytochalasin R (3.7 g) and the whole redissolved in ethyl acetate (25 ml). Cooling and filtration gave stout rhombs (1.6 g). Evaporation of the filtrate to 5 ml and then 1 ml gave further crystalline solid (0.45 g and 0.6 g respectively). The first two fractions were combined and recrystallised from methyl cyanide (× 3) to yield glistening solvated rods (1.4 g) of (19E)-21-*acetoxy*-6,7,13,14-*diepoxy*-18-*hydroxy*-16,18-*dimethyl*-10-*phenyl*[11]*cytochalas*-19-*ene*-1,17-*dione* (*cytochalasin R*) (**10**), m.p. 159—167 °C (Found: C, 68.1; H, 7.1; N, 5.0. $C_{30}H_{37}NO_7 \cdot CH_3CN$ requires C, 68.1; H, 7.1; N, 5.0%); *m/z* 523; $[\alpha]_D^{23}$ (AcOEt solvate) −73.1° (*c* 1.0 in $CHCl_3$), $[\alpha]_D^{23}$ (MeCN solvate) −49.3° (*c* 1.0 in MeCN); $\nu_{max.}$(KBr) 3 460, 3 315, 1 750, 1 712sh, and 1 700 cm^{-1}; δ_C 12.50, 19.55, 20.39, 20.48, and 24.77 (CH_3), 38.79 and 46.16 (CH_2), 37.45, 38.23, 44.78, 50.79, 54.75, 59.16, 61.79, 62.20, 76.16, 127.11, 129.03 × 2, 130.10 × 2, 130.56, and 132.22 (CH), and 55.20, 56.11, 78.76, 138.15, 170.61, 175.45, and 213.21 (C). Recrystallisation of the third fraction from methyl cyanide gave additional cytochalasin R (0.17 g). Evaporation of the m.l. and recrystallisation of the gummy residue from acetone–light petroleum (b.p. 80—100 °C) gave cytochalasin Q (0.23 g).

Tubes 40—60. These gave a viscous yellow gum (yellow spot) (7.1 g) which upon trituration with ethyl acetate and filtration after 12 h afforded a mixture of cytochalasin C, N, and O (0.26 g). Evaporation of the filtrate and crystallisation of the residue from acetone–light petroleum (b.p. 60—80 °C) gave (6S or 6R;13E,19E)-21-*acetoxy*-6,7,18-*trihydroxy*-16,18-*dimethyl*-10-*phenyl*[11]*cytochalasa*-13,19-*diene*-1,17-*dione* (*cytochalasin P*) (**8**) as needles (0.33 g), m.p. 169—173 °C (Found: C, 68.6; H, 7.5; N, 2.7. $C_{30}H_{39}NO_7$ requires C, 68.6; H, 7.4; N, 2.7%); *m/z* 525; $[\alpha]_D^{23}$ −35.8 (*c* 1.0 in MeOH); $\nu_{max.}$(KBr) 3 425, 1 747, 1 700, and 1 697 cm^{-1}; δ_C 13.42, 19.43, 20.63, 23.30, and 24.73 (CH_3), 38.73 and 46.26 (CH_2), 39.22, 42.72, 47.08, 51.82, 54.62, 76.81, 79.10, 126.67, 127.17, 128.80 × 2, 129.86 × 2, 131.06, 133.66, and 134.98 (CH), and 55.72, 76.08, 78.32, 139.04, 170.08, 175.59, and 211.56 (C). The compound separates as a gel from ethyl acetate.

Tubes 61—80. These gave a viscous yellow gum (pink spot) which was triturated with ethyl acetate and the mixture set aside (7 days). The resultant crystals were recovered, washed with ethyl acetate, and recrystallised from ethyl acetate to yield needles of punctaporonin B (**3**) (0.3 g), m.p. and mixed m.p. with an authentic sample 188 °C and having i.r. and ^1H n.m.r. spectra identical with the authentic compound.

A portion (0.5 g) of the combined solids comprising cytochalasin C, D, N, and O was applied to a column of SiO_2 (2 × 20 cm) in chloroform–methanol (95:5; 60 ml). Elution with the same solvent gave two fractions, one a mixture (0.24 g) of cytochalasin C, D, and N, the other (yellow spot) (6R or 6S;13E,19E)-21-*acetoxy*-6,7,18-*trihydroxy*-16,18-*dimethyl*-10-*phenyl*[11]*cytochalasa*-13,19-*diene*-1,17-*dione* (*cytochalasin O*) (**7**) as needles (32 mg), m.p. 258—265 °C from toluene–methanol (Found: C, 68.7; H, 7.6; N, 2.6. $C_{30}H_{39}NO_7$ requires

C, 68.5; H, 7.4; N, 2.7%); *m/z* 525; $[\alpha]_D^{23}$ −39.3° (*c* 1.0 in MeOH); $\nu_{max.}$(KBr) 3 410, 1 741, 1 603, and 1 691 cm^{-1}; δ_C 13.55, 19.44, 20.53, 24.68 and 25.30 (CH_3), 38.82 and 46.60 (CH_2), 39.85, 42.59, 44.73, 49.97, 54.18, 73.42, 78.77, 127.07, 127.46, 129.04 × 2, 130.09 × 2, 131.05, 133.49, and 134.59 (CH), 55.52, 72.56, 78.37, 138.43, 170.36, 175.66, and 211.39 (C).

A mixture of C, D, and N (0.5 g) in chloroform–butanol (60 ml; 97:3) was separated by flash chromatography to yield (a) *cytochalasin C* (0.22 g) (pale blue spot) as hair-like needles from methanol, m.p. 260—265 °C (Found: C, 70.7; H, 7.3; N, 2.6. Calc. for $C_{30}H_{37}NO_6$: C, 71.0; H, 7.35; N, 2.8%); *m/z* 507; $\nu_{max.}$(KBr) 3 402, 3 186, 1 740, 1 705, and 1 698sh; δ_H 1.05 (d, 3 H, *J* 6.3 Hz, 22-H_3), 1.41 (s, 3 H, 12-H_3), 1.58 (s, 3 H, 23-H_3), 1.95 (s, 1 H, 11-H_3), 1.93—1.96 (m, 1 H, 15α-H), 2.38 (s, 3 H, 21-OAc), 2.6—2.75 (m, 2 H, 15β-H and 16-H), 2.88 (d, 1 H, *J* 1.5 Hz, 4-H), 3.09 (dd, 1 H, *J* 10.0 and 10.0 Hz, 8-H), 3.16 (dd, 1 H, *J* 13.0 and 7.5 Hz, 10α-H), 3.22 (dd, 1 H, *J* 13.0 and 7.5 Hz, 10β-H), 3.69 (ddt, 1 H, *J* 7.5, 7.5, and 1.5 Hz, 3-H), 4.40—4.51 (m, 1 H, 7-H), 5.54 (ddd, 1 H, *J* 15.4, 10.0, and 5.5 Hz, 14-H), 5.66 (dd, 1 H, *J* 15.5 and 2.5 Hz, 19-H), 5.79 (d, 1 H, *J* 5.5 Hz, 7-OH), 6.02 (s, 1 H, 18-OH), 6.41 (dd, 1 H, *J* 2.5 and 2.5 Hz, 21-H), 6.59 (ddd, 1 H, *J* 15.4, 10.0 and 1.0 Hz, 13-H), 6.84 (dd, 1 H, *J* 15.5 and 2.5 Hz, 20-H), 7.22—7.34 (m, 5 H, Ar), and 9.41 (s, 1 H, NH); δ_C 14.92, 17.29, 19.49, 20.71, and 24.77 (CH_3), 38.72 and 45.35 (CH_2), 42.54, 50.71 × 2, 61.05, 69.13, 76.39, 127.04, 128.60, 129.07 × 2, 130.00 × 2, 132.78, 132.93, and 133.54 (CH), and 53.76, 78.62, 126.69, 134.06, 138.99, 170.93, 175.71, and 210.94 (C); (b) a mixture of cytochalasin C and D (45 mg), and (c) (7S,16S,18R,21R;13E,19E)-21-*acetoxy*-5,6-*epoxy*-7,18-*dihydroxy*-16,18-*dimethyl*-10-*phenyl*[11]*cytochalasa*-13,19-*diene*-1,17-*dione*, (*cytochalasin N*) (**6**) (37 mg, pink spot) as hair-like needles, m.p. 272 °C from methanol (Found: C, 69.1; H, 7.2; N, 2.6. $C_{30}H_{37}NO_7$ requires C, 68.8; H, 7.1; N, 2.7%); *m/z* 523; $[\alpha]_D^{23}$ −4° (*c* 0.5 in MeOH); $\nu_{max.}$(KBr) 3 410, 1 742, 1 705, and 1 699 cm^{-1}; δ_C 14.83, 19.40, 20.16, 20.74, and 24.64 (CH_3), 38.70 and 45.43 (CH_2), 42.42, 44.50, 49.02, 56.76, 70.15, 76.72, 127.04, 128.23, 129.03 × 2, 129.95 × 2, 131.18, 133.41, and 134.05 (CH), and 55.57, 63.42, 65.67, 78.45, 138.39, 170.73, 174.89, and 210.90 (C).

The mixture of cytochalasin C and D (45 mg) was chromatographed on a column of SiO_2 (2 × 10 cm) in chloroform–acetic acid (90:10) to yield cytochalasin C (9 mg) and *cytochalasin D* (13 mg) as hair-like needles from methanol, m.p. 267—270 °C (Found: C, 70.9; H, 7.4; N, 3.0. Calc. for $C_{30}H_{37}NO_6$ C, 71.0; H, 7.3; N, 2.8%); δ_C 13.71, 19.47, 20.63, and 24.71 (CH_3), 38.65, 45.58, and 112.26 (CH_2), 33.23, 42.54, 47.95, 50.14, 54.08, 71.34, 78.06, 126.88, 127.89, 128.86 × 2, 129.97 × 2, 132.25, 132.82, and 133.86 (CH), and 59.45, 78.46, 138.48, 151.64, 170.48, 175.13, and 210.91 (C).

Mycelium.—The mycelium (160 g) was extracted with chloroform (24 h). Evaporation yielded a solid (6.1 g) which was chromatographed as described above in chloroform–methanol (95:5) to yield cytochalasin C (820 mg) and a mixture of cytochalasin C, D, and N (280 mg) which was purified as described above from chloroform–butanol (97:3).

Cytochalasin N Acetate.—A suspension of cytochalasin N (0.1 g) in acetic anhydride (3 ml) and pyridine (0.5 ml) was set aside (3 h). The clear solution was poured into water and the oily suspension evaporated. Solution of the residual oil in cyclohexane (20 ml) and recrystallisation of the deposited solid from cyclohexane–acetone gave (7S,16S,18R,21R;13E,19E)-7,21-*diacetoxy*-5,6-*epoxy*-18-*hydroxy*-16,18-*dimethyl*-10-*phenyl*[11]-*cytochalasa*-13,19-*diene*-1,17-*dione* (*cytochalasin N acetate*) as rosettes of needles (58 mg), m.p. 187—192 °C (Found: C, 68.0; H, 7.0; N, 2.4. $C_{32}H_{39}NO_8$ requires C, 67.9; H, 6.9; N, 2.5%); *m/z* 565; $\nu_{max.}$(KBr) 3 480—3 400, 1 748, 1 742sh, 1 715, and 1 704 cm^{-1}; δ_H *inter alia* 2.1 (s, 3 H), 2.39 (s, 3 H), and 5.66 (d, 1 H, *J* 8.5 Hz, 7-H).

Deacetylcytochalasin N.—A suspension of cytochalasin N (100 mg) and potassium carbonate (70 mg) in methanol (2 ml) was set aside (2 h). The suspended potassium carbonate was filtered off and the solution acidified with acetic acid. Evaporation gave a gum which was dissolved in chloroform and the solution washed with water, dried, and evaporated. Dissolution of the resulting gum in acetone gave fine needles (63 mg) of (7S,16S,18R,21R;13E,19E)-5,6-*epoxy*-7,18,21-*trihydroxy*-16,18-*dimethyl*-10-*phenyl*[11]*cytochalasa*-13,19-*diene*-1,17-*dione* (*deacetylcytochalasin N*), m.p. 230—237 °C (Found: C, 70.1; H, 7.4; N, 2.8. $C_{28}H_{35}NO_5$ requires C, 69.85; H, 7.3; N, 2.9%); *m/z* 481; $\nu_{max.}$ 3 410, 1 703, and 1 695 cm^{-1}.

Sodium Borohydride Reduction of Cytochalasin N.—A mixture of sodium borohydride (50 mg) and cytochalasin N (50 mg) in ethanol (10 ml) was stirred for 1 h. The solution was diluted with water and the ethanol removed under reduced pressure. The aqueous solution was acidified and extracted with chloroform (3 × 5 ml) and the dried chloroform extract was evaporated. The solid residue was recrystallised from ethyl acetate–light petroleum (b.p. 60—80 °C) to yield needles (27 mg) of (7S,16S,18R,21R;13E,19E)-21-*acetoxy*-5,6-*epoxy*-7,17,18-*trihydroxy*-16,18-*dimethyl*-10-*phenyl*[11]*cytochalasa*-13,19-*dien*-1-*one* (17-*hydroxycytochalasin N*), m.p. 215—225 °C (Found: C, 68.4; H, 7.2; N, 2.2. $C_{30}H_{39}NO_7$ requires C, 68.55; H, 7.5; N, 2.7%); *m/z* 525; $\nu_{max.}$(KBr) 3 600—3 300, 1 744, and 1 695 cm^{-1}. T.l.c. in chloroform–methanol (95:5) indicated this to be a mixture of two isomers of close R_F.

Alkaline Degradation of Cytochalasin N.—A solution of cytochalasin N (320 mg) in a mixture of ethanol (15 ml) and sodium hydroxide solution (3M; 30 ml) was refluxed under nitrogen (1.5 h), and then cooled, and the ethanol removed under reduced pressure. The residual aqueous solution was acidified (2M HCl) and extracted with ethyl acetate (× 3), and the extract washed, dried, and evaporated to yield the crude acid (**12**) as a pale yellow oil (140 mg), *m/z* 399, soluble in all solvents except light petroleum.

A solution of the oil in tetrahydrofuran (THF; 5 ml) and 1M sulphuric acid (0.5 ml) was refluxed (1 h). The THF was removed under reduced pressure and the aqueous solution extracted with ethyl acetate (3 × 5 ml). The extract was washed dried, and evaporated to yield a brown gum which on trituration with chloroform gave a colourless solid (32 mg). Recrystallisation from acetone–light petroleum (b.p. 80—100 °C) gave 5-(1′-*benzyl*-5′,6′,7′-*trihydroxy*-6′,7′-*dimethyl*-3′-*oxoperhydroisoindol*-4′-*yl*)-2-*methylpenta*-4-*enoic acid* (**13**) as thin plates, m.p. 123—125 °C (Found: C, 65.9; H, 7.6; N, 3.3. $C_{23}H_{31}NO_6$ requires C, 66.2; H, 7.5; N, 3.4%); $\nu_{max.}$(KBr) 3 500—2 800 and 1 728—1 700 cm^{-1}; δ_H(270 MHz, numbering as for cytochalasin N), 1.40 (d, 3 H, *J* 6.6 Hz, 16-CH_3), 1.57 (s, 3 H, 11-H_3), 1.89 (s, 3 H, 12-H_3), 2.57 (dd, 1 H, *J* 14.6 and 7.6 Hz, 15α-H), 2.77—3.0 (m, 5 H, 15β-, 10α-, 10β-, 16-, and 4-H), 3.34 (dd, 1 H, *J* 7.6 and 7.1 Hz, 9-H), 3.5 (ddd, 1 H, *J* 10.8, 10.0, and 7.1 Hz, 8-H), 4.21 (dd, 1 H, *J* 7.3 and 5.6 Hz, 3-H), 4.66 (d, 1 H, *J* 10.8 Hz, 7-H), 4.8—6.2 (s, 4 H, 5-, 6-, 7-OH, CO_2H), 5.94 (ddd, 1 H, *J* 14.7, 7.8, and 6.1 Hz, 14-H), 7.2—7.38 (m, 5 H, Ar), 7.45 (dd, 1 H, *J* 14.7 and 10.0 Hz, 13-H), and 8.20 (s, 1 H, NH); δ_C[$(CD_3)_2CO$, 67.8 MHz] 16.66, 19.55, and 20.83 (CH_3), 37.14 and 41.19 (CH_2), 40.23, 42.38, 42.69, 45.41, 55.75, 71.09, 127.06, 129.15 × 2, 129.34, 130.28 × 2, and 134.36 (CH), and 75.89, 76.35, 139.58, 178.76, and 178.95 (C).

Aqueous Acid Hydrolysis of Cytochalasin N.—A solution of cytochalasin N (60 mg) in a mixture of THF (5 ml) and 1M

sulphuric acid (1 ml) was refluxed (1.5 h). It was then diluted with water (30 ml), the THF removed under reduced pressure, and the aqueous solution extracted with chloroform (3 × 10 ml). The washed and dried extract was evaporated and the residual gum dissolved in ethyl acetate (2 ml) to yield a solid which was purified by p.l.c. using chloroform–methanol (95:5) to yield unchanged cytochalasin N (upper band, 6 mg) and the 5,6-diol (**11**), (7S,16S,18R,21R;13E,19E)-21-*acetoxy*-5,6,7,18-*tetrahydroxy*-16,18-*dimethyl*-10-*phenyl*[11]*cytochalasa*-13,19-*diene*-1,17-*dione* (pink spot) as needles (23 mg) from ethyl acetate, m.p. 178 °C (Found: C, 66.2; H, 7.2; N, 2.5. $C_{30}H_{39}NO_8$ requires C, 66.5; H, 7.3; N, 2.6%); *m/z* 541; $\nu_{max.}$ 3 260—3 120, 1 745, 1 710, and 1 690sh cm^{-1}; δ_H 1.04 (d, 3 H, *J* 6.7 Hz, 22-H_3), 1.58 (s, 6-H, 23- and 12-H_3), 1.88 (s, 3 H, 11-H_3), 1.99 (dd, 1 H, *J* 5.0 and 12.0, 15α-H), 2.4 (s, 3 H, 21-OAc), 2.61—2.81 (m, 3 H, 4-, 15β-, and 16-H), 3.20—3.34 (m, 2 H, 8- and 10α-H), 3.95—4.15 (m, 1 H, 10β-H), 4.17 (dd, 1 H, *J* 9.0 and 7.0 Hz, 7-H), 4.40 (ddd, 1 H, *J* 8.5, 6.0, and 2.5 Hz, 3-H), 5.0 (s, 1 H, OH), 5.58 (ddd, 1 H, *J* 15.5, 10.5, and 5.0 Hz, 14-H), 5.75 (dd, 1 H, *J* 15.5 and 2.2 Hz, 19-H), 5.97 (s, 1 H, OH), 5.98 (s, 1 H, OH), 6.44 (s, 1 H, 21-H), 6.5 (br s, 1 H, OH), 6.75 (dd, 1 H, *J* 15.0 and 10.0 Hz, 13-H), 6.89 (d, 1 H, *J* 15.0 Hz, 20-H), 7.19—7.31 (m, 5 H, Ar), and 8.79 (s, 1 H, NH); δ_C($CDCl_3$, 67.8 MHz) 19.03, 20.40 (br), 21.01, 21.97 (br), and 23.55 (CH_3), 36.12 and 44.64 (CH_2), 42.58, 44.71, 54.57, 57.21, 75.78, 76.10 (br), 126.70, 127.24, 128.71 × 2, 129.09 × 2, 129.48 (br), 132.81, and 133.90 (br) (CH), and 53.45, 74.93, 76.32, 78.27, 138.19, 170.31, 176.94, and 212.67 (C).

The acetate of (**11**), (7S,16S,18R,21R;13E,19E)-7,21-*diacetoxy*-5,6,18-*trihydroxy*-16,18-*dimethyl*-10-*phenyl*[11]*cytochalasa*-13,19-*diene*-1,17-*dione* was prepared as described for cytochalasin N acetate and crystallised from acetone–light petroleum (b.p. 60—80 °C) as rhombs, m.p. 160—164 °C (Found: C, 66.1; H, 7.2; N, 2.4. $C_{32}H_{41}NO_9$ requires C, 65.8; H, 7.0; N, 2.4%); *m/z* 583; $\nu_{max.}$($CHCl_3$) 3 420, 1 738, 1 728, and 1 704 cm^{-1}; δ_H($CDCl_3$, 270 MHz) *inter alia* 1.98 (s, 3 H, OAc), 2.27 (s, 3 H, OAc), 4.63 (d, 1 H, *J* 10.8 Hz, 7-H); δ_C($CDCl_3$, 67.8 MHz) 19.40, 20.86, 21.15, 22.53 (br), 22.69 (br), and 24.29 (CH_3), 38.18 and 42.82 (CH_2), 42.82, 45.18, 56.11, 75.83, 76.07, 126.92, 127.57, 128.88 × 2, 129.42 × 2, 130.57, 132.40, and 132.64 (CH), and 53.99, 76.37, 77.86, 80.01 (br), 138.02, 170.11, 171.71, 175.09, and 210.38 (C).

Boron Trifluoride Isomerisation of Cytochalasin N.—Boron trifluoride–diethyl ether (10 μl) was added to a suspension of cytochalasin N (200 mg) in methyl cyanide (distilled over P_2O_5, 5 ml): dissolution occurred during 5 h. The solution was set aside (48 h), then diluted with water (5 ml), and evaporated to 5 ml under reduced pressure. Extraction with chloroform and evaporation of the dried chloroform extract gave a gummy solid which was applied to a column of SiO_2 (10 × 1.5 cm) in chloroform–methanol (95:5) and eluted with the same solvent to yield two fractions. The most mobile fraction (blue spot) gave a gum yielding (13E,19E)-7,8-(21-*acetoxy*-18-*hydroxy*-16,18-*dimethyl*-17-*oxocycloundec*-13,19-*dienyl*)-3-*benzyl*-6-*formyl*-5-*hydroxy*-5,6-*dimethylhexahydrocyclopenta*[c]*pyrrol*-1(2H)-*one* (**6a**) as small rhombs (19 mg), m.p. 119—121 °C from ethyl acetate–cyclohexane (Found: C, 71.2; H, 7.5; N, 2.7. $C_{30}H_{37}NO_6$ requires C, 71.0; H, 7.35; N, 2.8%); *m/z* 523; $\nu_{max.}$($CHCl_3$) 3 420, 1 740sh, 1 735, 1 707, and 1 702 cm^{-1}; δ_H(270 MHz) 1.11 (d, 3 H, *J* 6.8 Hz, 22-H_3), 1.36 (s, 3 H, 10- or 11- or 23-H_3), 1.39 (s, 3 H, 23- or 11- or 10-H_3), 1.68 (s, 3 H, 11- or 23- or 10-H_3), 2.03 (dd, 1 H, *J* 12.8 and 4.0 Hz, 15α-H), 2.35 (s, 3 H, 21-OAc), 2.64 (ddd, 1 H, *J* 12.8, 11.7, and 10.6 Hz, 15β-H), 2.98 (d, 1 H, *J* 2.6 Hz, 4-H), 2.90—3.05 (m, 2 H, 9α- and 16-H), 3.14 (dd, 1 H, *J* 13.2 and 7.0 Hz, 9β-H), 3.94 (d, 1 H, *J* 10.6 Hz, 7-H), 4.11 (ddd, 1 H, *J* 7.3, 6.9, and 2.6 Hz, 3-H), 5.44 (ddd, 1 H, *J* 15.0, 11.4 and 4.0 Hz, 14-H), 6.02 (dd, 1 H, *J* 15.8 and 2.0 Hz, 19-H), 6.09 (s, 1 H, OH), 6.36 (m, 1 H, 21-H), 6.39 (dd, 1 H, *J* 15.0 and 10.6 Hz, 13-H), 6.41 (s, 1 H, OH), 7.00 (dd, 1 H, *J* 15.8 and 3.5 Hz, 20-H), 7.16—7.38 (m, 5-H, Ar), 9.37 (s, 1 H, NH), and 10.06 (s, 1 H, 12-H); δ_C($CDCl_3$, 67.8 MHz) 13.46, 19.19, 19.74, 21.26, and 23.87 (CH_3), 38.20 and 45.13 (CH_2), 42.69, 52.75, 55.09, 59.52, 72.97, 126.01, 127.06, 128.11, 128.88 × 2, 129.31 × 2, 131.40, 135.86, and 203.99 (CH), and 59.86, 64.60, 77.95, 80.75, 136.89, 169.85, 173.82, and 211.75 (C). The second fraction (orange–red spot) gave the (13E,19E)-21-*acetoxy*-5,18-*dihydroxy*-16,18-*dimethyl*-10-*phenyl*[11]*cytochalasa*-13,19-*diene*-1,7,17-*trione* (**6b**) as a chromatographically homogeneous gummy solid (36 mg), *m/z* 523; $\nu_{max.}$($CHCl_3$) 3 408, 1 738, and 1 711 cm^{-1}; δ_H(270 MHz) 1.02 (d, 3 H, *J* 5.9 Hz, 22-H_3), 1.18 (s, 3 H, 11-H_3), 1.34 (d, 3 H, *J* 7.0 Hz, 12-H_3), 1.56 (s, 3 H, 23-H_3), 2.00 (dd, 1 H, *J* 10.8 and 5.6 Hz, 15α-H), 2.36 (s, 3 H, 21-OAc), 2.41 (q, 1 H, *J* 7.0 Hz, 6-H), 2.68—2.78 (m, 2 H, 16- and 15β-H), 2.94 (d, 1 H, *J* 2.2 Hz, 4-H), 3.11—3.26 (ddd, 2 H, *J* 13.4, 7.2, and 6.2 Hz, 10α- and 10β-H), 3.97 (ddd, 1 H, *J* 7.1, 6.2 and 2.2 Hz, 3-H), 5.13 (d, 1 H, *J* 9.6 Hz, 8-H), 5.39—5.49 (ddd, 1 H, *J* 15.4, 10.6, and 5.6 Hz, 14-H), 5.74 (dd, 1 H, *J* 15.8 and 2.4 Hz, 19-H), 6.02 (s, 1 H, OH), 6.42 (dd, 1 H, *J* 2.4 and 2.4 Hz, 21-H), 6.60 (dd, 1 H, *J* 16.0 and 9.6 Hz, 13-H), 6.68 (s, 1 H, OH), 6.87 (dd, 1 H, *J* 15.8 and 2.4 Hz, 20-H), 7.23—7.45 (m, 5 H, Ar), and 9.61 (s, 1 H, NH); δ_C(67.8 MHz) 9.81, 19.38, 21.03, 24.64, and 25.50 (CH_3), 38.96 and 46.13 (CH_2), 42.33, 48.64, 54.32, 56.55, 57.31, 76.65, 127.07, 128.58, 129.04 × 2, 129.41, 130.26 × 2, 133.31, and 133.96 (CH), and 55.35, 72.76, 78.69, 138.49, 171.16, 175.20, 210.95, and 211.94 (C).

Alkaline Hydrolysis of Cytochalasin O.—A solution of cytochalasin O (100 mg) in a mixture of ethanol (15 ml) and 3M aqueous sodium hydroxide (15 ml) was refluxed (1.5 h) under nitrogen. The orange coloured mixture was cooled, the alcohol evaporated under reduced pressure, and the product isolated as described for the hydrolysis of cytochalasin N. The product was purified by t.l.c. with ether–light petroleum (b.p. 60—80 °C)–acetic acid (60:40:5) to yield a gum (32 mg) which failed to crystallise. Acid treatment and isolation of the product as previously described for cytochalasin N gave needles, m.p. 165 °C of the acid 5-(1′-*benzyl*-5′,6′-*dihydroxy*-6′,7′-*dimethyl*-3′-*oxoperhydroisoindol*-4′-*yl*)-2-*methylpent*-4-*enoic acid* (**14**) from acetone–light petroleum (b.p. 60—80 °C) (Found: C, 68.5; H, 7.7; N, 3.2. $C_{23}H_{31}NO_5$ requires C, 68.8; H, 7.8; N, 3.5%); $\nu_{max.}$($CHCl_3$) 3 560—2 800 and 1 730 cm^{-1}; δ_H($CDCl_3$, 270 MHz, numbering as for cytochalasin O) 0.89 (d, 3 H, *J* 7.3 Hz, 11-H_3), 1.18 (d, 3 H, *J* 7.0 Hz, 16-CH_3), 1.27 (s, 3 H, 12-H_3), 2.02 (dq, 1 H, *J* 7.3 and 4.2 Hz, 5-H), 2.16 (dd, 1 H, *J* 12.3, 11.5 Hz, 15α-H), 2.30—2.45 (m, 3 H, 8-, 9-, and 15β-H), 2.5—2.7 (m, 3 H, 10α-, 10β-, and 16-H), 2.93 (dd, 1 H, *J* 13.9 and 4.2 Hz, 4-H), 3.14 (d, 1 H, *J* 9.7 Hz, 7-H), 3.56 (ddd, 1 H, *J* 9.3, 9.3, and 4.2 Hz, 3-H), 5.40 (dd, 1 H, *J* 15.2 and 9.0 Hz, 13-H), 5.67 (dt, 1 H, *J* 15.2 and 7.5 Hz, 14-H), 6.27 (s, 1 H, NH or OH), and 7.1—7.4 (m, 5 H, Ar); δ_C($CDCl_3$, 67.8 MHz) 10.27, 16.85, and 25.91 (CH_3), 36.84 and 39.99 (CH_2), 37.87, 39.91, 42.74, 45.94, 46.52, 55.81, 74.71, 126.93, 128.85 × 2, 128.95 × 2, 131.24, and 131.46 (CH), and 73.95, 137.47, 176.86, and 179.23 (C).

Sodium Periodate Oxidation of Cytochalasin O.—A solution of sodium periodate (60 mg) in water (2.5 ml) was added to a solution of cytochalasin O (100 mg) in methanol (10 ml) and the mixture set aside (2.5 h). The solution was evaporated to near dryness, diluted with water (2 ml) and extracted with chloroform. Evaporation of the dried chloroform extract gave a solid which was dissolved in ethyl acetate. After 24 h the crystalline unchanged cytochalasin O was filtered off, the filtrate evaporated, and the residue crystallised from ether–light petroleum (b.p. 60—80 °C) to yield 2-*acetoxy*-4′-(1″-*acetylethyl*)-5′-*benzyl*-11-*formyl*-5-*hydroxy*-5,7-*dimethylcycloundeca*-3,9-*diene*-3′-*pyrrolidine*-2′,6-*dione* (**15**) as needles (17 mg), m.p. 120—122 °C (Found: C, 68.9; H, 7.3; N, 2.6. $C_{30}H_{37}NO_7$ requires C, 68.8; H,

7.1; N, 2.7%); *m/z* 523; $\nu_{max.}$(KBr) 3 440, 1 745, 1 735vw, and 1 710 cm^{-1}; δ_H($CDCl_3$, 270 MHz) 1.19 (d, 3 H, *J* 5.9 Hz, 13-H_3), 1.22 (d, 3 H, *J* 5.9 Hz, 2″-H_3), 1.50 (s, 3 H, 14-H_3), 1.98 (dd, 1 H, *J* 12.4 and 2.9 Hz, 8α-H), 2.20 (m, 1 H, 4′-H), 2.24 (s, 3 H, Ac or OAc), 2.26 (s, 3 H, OAc or Ac), 2.47 (dd, 1 H, *J* 13.4 and 10.3 Hz, 6′α-H), 2.55 (m, 1 H, 8β-H), 2.65—2.85 (m, 2 H, 1″- and 7-H), 3.15 (dd, 1 H, *J* 2.9 and 13.4 Hz, 6′β-H), 3.51 (ddd, 1 H, *J* 9.3, 5.8, and 2.0 Hz, 5′-H), 3.61 (dd, 1 H, *J* 5.9 and 2.0 Hz, 11-H), 4.97 (dd, 1 H, *J* 2.4 and 15.8 Hz, 4-H), 5.49 (m, 2 H, 9- and 10-H), 5.55 (dd, 1 H, *J* 2.4 and 2.6 Hz, 22-H), 5.67 (s, 1 H, 1′-NH or 5′-OH), 6.16 (dd, 1 H, *J* 15.8 and 2.6 Hz, 3-H), 7.1—7.34 (m, 5 H, Ar), and 9.30 (d, 1 H, *J* 2.0 Hz, 12-CHO); δ_C($CDCl_3$, 67.8 MHz) 19.17, 19.42, 21.10, 24.23, and 31.4 (CH_3), 37.03 and 44.05 (CH_2), 42.20, 46.60, 53.32, 57.83, 58.95, 79.06, 125.78, 127.03, 127.78, 128.95 × 2, 129.01 × 2, 132.00, 135.21, and 198.62 (CH), and 55.17, 77.73, 137.71, 169.06, 173.40, 209.43, and 211.07 (C).

Oxidation of Cytochalasin C with m-*Chloroperbenzoic Acid.*—*m*-Chloroperbenzoic acid (80—90%; 210 mg) was added to a solution of cytochalasin C (500 mg) in dichloromethane (40 ml). After 24 h the solution was washed with saturated aqueous sodium hydrogen carbonate, dried, and evaporated to yield a solid (0.49 g) which was dissolved in chloroform–butanol (97:3; 10 ml) and column (20 × 1.5 cm) chromatographed using the same solvent mixture to yield four components which were eluted in the following order. (*a*) Unchanged cytochalasin C (5 mg); (*b*) cytochalasin N (210 mg) crystallising from methanol as needles (m.p. 272 °C) and having ^1H and ^{13}C n.m.r. spectra identical with those of the naturally occurring compound; (*c*) a solid (pink spot) yielding needles (5 mg) of *isocytochalasin N* (**18**), m.p. 248—255 °C from methanol (Found: C, 68.8; H, 7.1; N, 2.6. $C_{30}H_{37}NO_7$ requires C, 68.8; H, 7.1; N, 2.7%); *m/z* 523; $\nu_{max.}$(KBr) 3 420, 1 740, and 1 708 cm^{-1}; δ_H 0.99 (s, 3 H, 12-H_3), 1.04 (d, 3 H, *J* 6.5 Hz, 22-H_3), 1.57 (s, 3 H, 23- or 11-H_3), 1.63 (s, 3 H, 11- or 23-H_3), 1.97 (dd, 1 H, *J* 12.5 and 4.7 Hz, 15α-H), 2.47 (s, 3 H, OAc), 2.58 (d, 1 H, *J* 1.5 Hz, 4-H), 2.63 (dd, 1 H, *J* 11.0 and 12.5 Hz, 15β-H), 2.73 (m, 1 H, 16-H), 3.06 (dd, 1 H, *J* 11.0 and 10.0 Hz, 8-H), 3.20 (dd, 1 H, *J* 13.0 and 9.0 Hz, 10α-H), 3.29 (dd, 1 H, *J* 13.0 and 7.0 Hz, 10β-H), 4.18 (d, 1 H, *J* 11.0 Hz, 7-H), 4.23 (ddd, *J* 9.0, 7.0, and 1.5 Hz, 3-H), 5.59 (ddd, 1 H, *J* 15.0, 10.0, and 5.0 Hz, 14-H), 5.65 (dd, 1 H, *J* 15.0 and 2.5 Hz, 19-H), 6.36 (dd, 1 H, *J* 2.5 and 2.5 Hz, 21-H), 6.44 (ddd, *J* 15.0, 10.0, and 1.0 Hz, 13-H), 6.74 (dd, 1 H, *J* 15.0 and 2.5 Hz, 20-H), 7.22—7.36 (m, 5 H, Ar), and 9.37 (d, 1 H, *J* 2 Hz, NH); δ_C(67.8 MHz) 16.04, 19.11, 19.49, 20.75, and 24.77 (CH_3), 38.72 and 44.35 (CH_2), 42.49, 48.07, 48.31, 58.61, 70.09, 76.70, 127.05, 128.77, 129.11 × 2, 130.01 × 2, 132.46, 132.90, and 133.14 (CH), and 53.37, 64.89, 69.58, 78.58, 138.91, 171.00, 175.47, and 210.65 (C); and (*d*) a solid (46 mg) (red spot) yielding needles from ethanol of (7S,16S,18R,21R;13E)-21-*acetoxy*-5,6,19,20-*diepoxy*-7,18-*dihydroxy*-16,18-*dimethyl*-10-*phenyl*[11]*cytochalas*-13-*ene*-1,17-*dione* (*cytochalasin C* 5,6,19,20-*diepoxide*) (**16**), m.p. 265—275 °C (Found: C, 66.8; H, 6.9; N, 2.6. $C_{30}H_{37}NO_8$ requires C, 66.8; H, 6.9; N, 2.6%); *m/z* 539; $\nu_{max.}$(KBr) 3 460, 1 742, 1 712, and 1 699 cm^{-1}; δ_H 1.04 (d, 3 H, *J* 6.5 Hz, 22-H_3), 1.18 (s, 3 H, 12-H_3), 1.57 (s, 3 H, 23- or 11-H_3), 1.65 (s, 3 H, 11- or 23-H_3), 2.04 (ddd, 1 H, *J* 12.0, 5.0, and 3.0 Hz, 15α-H), 2.19 (s, 3 H, 21-OAc), 2.72 (ddd, 1 H, *J* 12.0, 11.5, and 11.5 Hz, 15β-H), 2.98 (d, 1 H, *J* 2.0 Hz, 4-H), 3.13—3.26 (m, 3 H, 10α-, 10β-, and 16-H), 3.62 (dd, 1 H, *J* 10.5 and 10.5 Hz, 8-H), 3.79 (d, 1 H, *J* 2.0 Hz, 19-H), 4.05 (ddt, 1 H, *J* 9.0, 7.0, and 2.0 Hz, 3-H), 4.16 (dd, 1 H, *J* 10.5 and 6.5 Hz, 7-H), 4.19 (dd, 1 H, *J* 2.0 and 1.0 Hz, 20-H), 5.63 (s, 1 H, 18-OH), 5.82 (ddd, 1 H, *J* 15.5, 10.0, and 5.5 Hz, 14-H), 6.11 (s, 1 H, 21-H), 6.16 (d, 1 H, *J* 6.5 Hz, 7-OH), 6.80 (dd, 1 H, *J* 15.5 and 10.5 Hz, 13-H), 7.23—7.43 (m, 5 H, Ar), and 9.70 (s, 1 H, 2-NH); δ_C(67.8 MHz) 14.92, 19.11, 20.12, 20.63, and 22.60 (CH_3), 38.90 and 45.49 (CH_2), 41.99, 43.94, 49.76, 54.13, 57.18, 60.68, 70.17, 74.19, 127.13, 129.09 × 2, 130.18 × 2, 132.10, and 132.78 (CH), and 55.06, 63.56, 65.85, 77.30, 138.31, 171.01, 174.91, and 216.26 (C).

Aqueous Acid Hydrolysis of Isocytochalasin N.—Hydrolysis of isocytochalasin N (20 mg) as described for cytochalasin N gave a 5,6-diol, m.p. 178 °C (Found: C, 66.25; H, 7.6; N, 2.5. Calc. for $C_{20}H_{39}NO_8$ C, 66.5; H, 7.25; N, 2.6%). The ^1H and ^{13}C n.m.r. spectra were identical with those of the diol from cytochalasin N.

Oxidation of Cytochalasin D (**5**) *with* m-*Chloroperbenzoic Acid.*—A mixture of *m*-chloroperbenzoic acid (105 mg) and cytochalasin D (250 mg) in dichloromethane (20 ml) was set aside overnight. The solution was then washed with aqueous sodium hydrogen carbonate, dried, and evaporated and the residue separated by p.l.c. in chloroform–butanol (97:3). The major component was a solid (blue spot 86 mg) yielding solvated needles of cytochalasin D 13,14-epoxide, (7S,16S,18R,21R;19E)-21-*acetoxy*-13-*epoxy*-7,18-*dihydroxy*-16,18-*dimethyl*-10-*phenyl*[11]*cytochalasa*-6(12),19-*diene*-1,17-*dione* (**17**), m.p. 128—130 °C from cyclohexane; *m/z* 323; the solvent could not be removed without heating the compound above its m.p. under reduced pressure; $\nu_{max.}$(KBr) 3 430, 1 742, and 1 704 cm^{-1}; δ_H(270 MHz) 0.74 (d, 3 H, *J* 6.8 Hz, 11-H_3), 1.09 (d, 3 H, *J* 6.8 Hz, 22-H_3), 1.63 (s, 3 H, 23-H_3), 1.96—2.15 (m, 2 H, 15α- and 15β-H), 2.31 (dd, 1 H, *J* 8.8 and 8.8 Hz, 8-H), 2.39 (s, 3 H, 21-OAc), 2.46 (dd, 1 H, *J* 5.5 and 2.7 Hz, 4-H), 2.84—2.91 (m, 2 H, 5- and 10α-H), 2.96—3.1 (m, 3 H, 10β-, 14-, and 16-H), 3.61 (m, 1 H, 3-H), 4.44 (dd, 1 H, *J* 8.4 and 2.2 Hz, 13-H), 4.80 (d, 1 H, *J* 9.2 Hz, 7-H), 5.02 (s, 1 H, 12α-H), 5.05 (s, 1 H, 7- or 18-OH), 5.35 (s, 1 H, 12β-H), 6.14 (dd, 1 H, *J* 2.6 and 2.4 Hz, 21-H), 6.20 (dd, 1 H, *J* 15.8 and 2.6 Hz, 19-H), 6.62 (br s, 1 H, 18- or 7-OH), 7.18 (dd, 1 H, *J* 15.8 and 2.4 Hz, 20-H), 7.22—7.35 (m, 5 H, Ar), and 9.35 (s, 1 H, 2-NH); δ_C(67.8 MHz) 13.08, 20.42, 20.56, and 24.89 (CH_3), 38.52, 45.30, and 112.23 (CH_2), 32.68, 38.98, 45.08, 50.27, 54.38, 59.41, 61.21, 74.38, 77.07, 127.00, 128.96 × 2, 130.02 × 2, 131.00, and 132.41 (CH), and 53.75, 78.68, 138.44, 149.31, 170.74, 175.06, and 213.18 (C).

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