Cytochalasins E and K, Toxic Metabolites from *Aspergillus clavatus*

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The isolation of cytochalasin E and cytochalasin K from *Aspergillus clavatus* is described. The structure of cytochalasin K, a new metabolite, has been determined by spectroscopic data and chemical reactions. The relationship between cytochalasin E and cytochalasin K was confirmed by chemical conversion. Chemical reactions on cytochalasins E and K, both of which contain the unique vinyl carbonate moiety, are described.

ASPERGILLUS CLAVATUS Desm., a fungus encountered on stored grain and cereal products sporadically reaches high levels of infestation in grain sorghum during the malting process. Under experimental conditions, cultures of *A. clavatus* have been found to be acutely toxic to ducklings and rats. A toxinogenic strain, *A. clavatus* MRC 1181, isolated from sorghum malt was grown on sterilized whole maize and the toxic principles removed by prolonged extraction with chloroform–methanol. Fractionation of the toxic extract yielded cytochalasin E (1) and cytochalasin K (2), which accounted for the toxigenicity of this strain of *A. clavatus*.

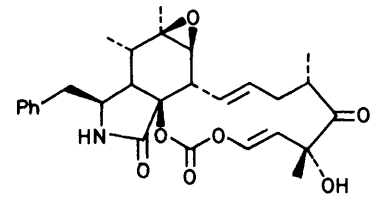
This paper relates the physical and chemical properties of cytochalasin E and K and is the first report on the natural occurrence of cytochalasin K. The structure of cytochalasin E (1) has been reported by two different groups.[1,2]

Cytochalasin K, $C_{28}H_{33}NO_7$, crystallised from hexane–acetone as white crystals, m.p. 246—248 °C. Structure (2) was assigned to cytochalasin K on the basis of its physicochemical properties. A strong absorption ($\nu_{max.}$ 1 765 cm^{-1}) in the i.r. spectrum of (2) indicated the presence of a vinyl carbonate moiety[3]; in cytochalasin E this grouping gives rise to absorption at 1 762 cm^{-1}. The peak at 1 729 cm^{-1} was assigned to the 17-ketone and 1-lactam carbonyl absorption of (2). A peak at 1 662 cm^{-1} was assigned to the 19,20-double bond; this absorption is absent in the spectrum of compound (9). Strong u.v. absorption was displayed by cytochalasin E [$\lambda_{max.}$ (MeOH) 263, 257, and 252 nm (log ε 3.59, 3.78, and 3.73)] and cytochalasin K, whereas other cytochalasins, *e.g.* cytochalasin B, showed weak absorption in this area owing to the phenyl moiety only. This strong absorption was absent in the u.v. spectrum of 19,20-dihydrocytochalasin E (9); it had u.v. properties similar to those of cytochalasin B. The above-mentioned prominent absorption can therefore be ascribed to the vinyl carbonate unit present in cytochalasins E and K.

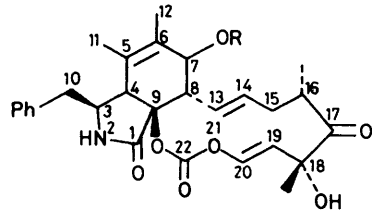
The ^1H n.m.r. spectrum of cytochalasin K (2) was analysed with the aid of proton-proton spin decoupling experiments (see Experimental section for assignments). The spectrum shows the presence of two *trans*-disubstituted double-bonds. Two sharp doublets at δ 5.57 and 6.57 ($J_{19,20}$ 13 Hz) were assigned to H-19 and H-20, respectively, whereas H-13 and H-14 resonate as multiplets at δ 6.21—6.05 and δ 5.47—5.20 ($J_{13,14}$ 13 Hz), respectively. The proton at C-3 appears as a triplet (δ 3.38, J 8 Hz). The multiplicity of H-3 is different from that of H-3 in cytochalasin E (1) (m, δ 3.70), but is similar to that of H-3 in cytochalasin C[4], a compound which contains the same partial structure as proposed for cytochalasin K. The resonances of the methyl groups define the position of the fully substituted double-bond in cytochalasin K. One three-proton doublet (δ 1.10, J 6 Hz) and three three-proton singlets (δ 1.44, 1.47 and 1.61) in the spectrum are assigned to the one secondary methyl and the three tertiary methyl groups, respectively, thus confirming the presence of the 5,6-double bond.

The presence of one secondary hydroxy-group in cytochalasin K was substantiated by acetylation. The ^1H n.m.r. spectrum of the acetate (3) shows the presence of one acetate group (δ 1.95); in addition H-7 (δ 5.35) experienced a strong downfield shift (Δδ 1.55) upon acetylation. The coupling constant for H-7 ($J_{7,8}$ 9 Hz) is consistent with a dihedral angle of 180 °[5] and defines the stereochemistry at C-7 as shown in compounds (2) and (3). Oxidation of cytochalasin K with chromium trioxide in pyridine afforded the α,β-unsaturated ketone (4).

The structure of cytochalasin K was confirmed by an acid-catalysed rearrangement of cytochalasin E (1). Two products were obtained, one of which was identical with cytochalasin K. The ^1H n.m.r. spectrum of the second compound shows the presence of an exocyclic methylene group (s, δ 5.40 and 5.17, 2 H), a tertiary methyl group (δ 1.50) and two secondary methyl groups (δ 1.30 and 1.15, d, J 6 Hz). Compound (5) was, therefore, identified as the $\Delta^{6,12}$-isomer of cytochalasin K. The ease of rearrangement of cytochalasin E (1) raised the question of whether cytochalasin K (2) was a natural product or an artefact obtained during the isolation process. The fact that only cytochalasin K (2) and not the $\Delta^{6,12}$-isomer (5) was isolated suggested that cytochalasin K is a natural product. This was confirmed by extraction of the mouldered maize at room temperature with acetone in a Waring blender. Cytochalasin E was found to be stable under these conditions and cytochalasin K was obtained in the extract. The perhydroisoindole unit present in cytochalasin E and K is identical with that in chaetoglobosins A and B, two cytotoxic metabolites isolated by Sekita *et al.*[6] from *Chaetomium globosum*.



(1)



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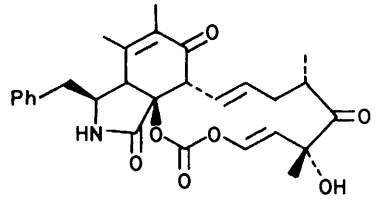
(3) R = Ac



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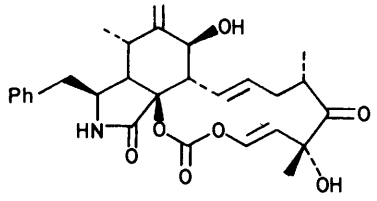

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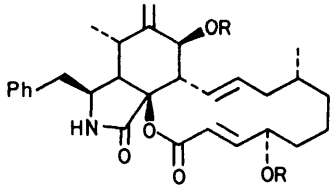

(6) R = H
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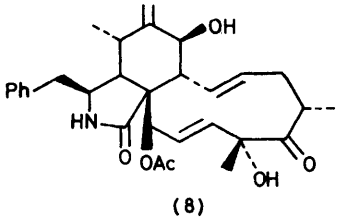

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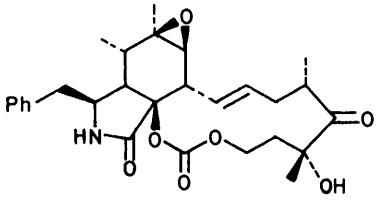


(9)

Assignments of the ^{13}C n.m.r. spectra of cytochalasin E (1) and 7-*O*-acetylcytochalasin K (3) derived from known chemical shifts are based on a comparison with ^{13}C n.m.r. data reported for cytochalasins B (6) and D (8).[7] The chemical shifts, together with the multiplicities derived from off-resonance decoupling experiments are collated in the Table. The assignments for cytochalasin E (1) and 7-*O*-acetylcytochalasin K (3) reflect the expected shift differences due to differences in structure; the signal due to C-19 appears at δ 120.3 and 120.5, respectively. C-20 Is bonded to oxygen and is, therefore, expected to resonate at rather low field; it appears at δ 142.0 and 142.2 for compounds (1) and (3), respectively; the oxygen-bearing vinyl carbon atom in aflatoxin B_1 resonates at δ 145.4.[8] It is important to note that C-22, belonging to the unique vinyl carbonate moiety appears at δ 149.2 and δ 149.0 in compounds (1) and (3) respectively and that this resonance shifts downfield to δ 152.8 in 19,20-dihydrocytochalasin E (9). This is to our knowledge the first ^{13}C n.m.r. assignment for a cyclic vinyl carbonate moiety.

TABLE

^{13}C N.m.r. data of cytochalasin E (1), 7-*O*-acetylcytochalasin K (3), 19,20-dihydrocytochalasin E (9), 7,20-di-*O*-acetylcytochalasin B (7) and cytochalasin D (8)

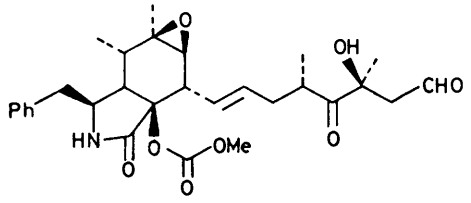
Carbon atom	(1) δ *	(3) δ *	(9) δ *	(7) δ *	(8) [7] δ †
1	170.7s	170.0s	171.6t	170.9s	174.9
3	53.7d	59.1d	53.7d	52.9d	54.0
4	47.2d	47.8d	47.5d	47.5d	50.0
5	35.9d	127.5s ‡	36.5d	32.9d	33.1
6	57.4s	128.8s ‡	57.2s	145.5s	151.4
7	60.7d	72.2d	60.7d	71.9d	71.2
8	45.8d	47.4d	45.3d	45.5d	47.8
9	87.4s	85.9s	85.5s	83.0s	54.4
10	44.2t	43.8t	44.7t	43.1s	45.5
11	13.1q	14.2q	12.3q	13.3q	13.6
12	20.1q	20.6q	21.2q	115.1t	112.2
13	128.7d	129.5d	127.8d	126.6d	132.1
14	131.4d	131.4d	132.8d	135.1d	132.7
15	39.2t	39.1t	37.8t	41.2t	38.6
16	40.8d	40.9d	39.6d	34.6d	42.4
17	211.9s	211.5s	214.4s	31.7t	210.7
18	76.7s	77.1s	75.9s	20.6t	78.3
19	120.3d	120.5d	36.2t	31.6t	127.7
20	142.0d	142.4d	63.2t	70.8d	133.7
21				147.4d	77.9
22	149.2s	149.0s	152.8s	119.4d	
23				163.7s	
1′	135.8s	136.5s	136.8s	136.5s	138.3
2′,6′	129.9d	129.8d	129.1d	129.3d	129.9
3′,5′	128.8d	128.9d	128.8d	128.3d	128.7
4′	127.2d	127.2d	126.9d	126.9d	126.8
16-Me	19.6q	20.1q	19.0q	19.9q	19.44
18-Me	24.4q	24.6q	28.0q		24.64
*C*OCH$_3$		170.0s		169.5s	170.3
				169.2s	
CO*C*H$_3$		17.8q		20.6q	20.6
				20.6q	

* In $CDCl_3$ relative to internal Me_4Si. Letters refer to multiplicities observed upon off-resonance decoupling. † In [$^2H_5$]pyridine relative to internal Me_4Si. ‡ Assignments may be interchanged.

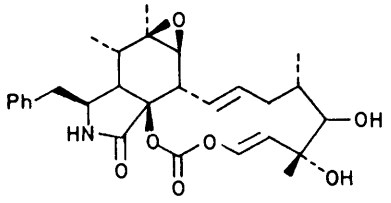
The difference in structure in cytochalasin E (1) and 7-*O*-acetylcytochalasin K (3) are reflected in resonances of the perhydroisoindole moiety. In cytochalasin E (1), C-5 appears as a doublet at δ 35.9, whereas the oxiran carbon atoms (C-6 and C-7) resonate at δ 57.4 and 60.7, respectively. The signal due to C-7 of 7-*O*-acetylcytochalasin K (3) appears at δ 72.7. The resonances corresponding to C-5 and C-6 overlap with the aromatic signals and could not be distinguished.

Since cytochalasins E and K are the only known com-

pounds containing a vinyl carbonate moiety, the chemistry of cytochalasin E (1) was investigated. Hydrogenation over Pd–C in ethyl acetate yielded only the 19,20-dihydro-compound (9). This substance was characterised by the appearance of H-19 and H-20 as two-proton multiplets at δ 4.20 and 2.20, respectively.



(10)



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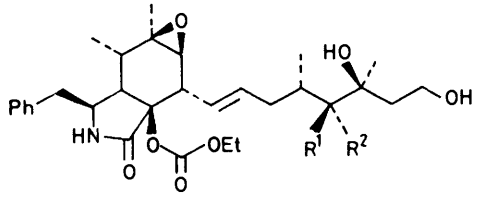
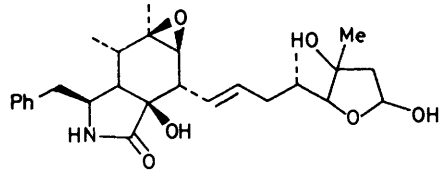


(12) R^1 =OH, R^2 = H
(13) R^1 = H, R^2 = OH



(14)

Treatment of cytochalasin E (1) with alkaline methanol led to the formation of the aldehyde methyl ester (10). The ^{1}H n.m.r. spectrum of this compound lacked the signals due to the vinyl ether moiety and indicated the formation of a CH_2CHO grouping (δ 3.25, dd; δ 9.80, s; δ 3.25, dd) and a methyl ester (δ 3.90, s).

Three products were obtained upon reduction of cytochalasin E (1) with sodium borohydride. The major compound was identified as the 17-hydroxy-derivative (11). The two minor compounds, (12) and (13), which had identical mass spectra, originated from the reduction of the 17-keto-group and ring opening of the macrocyclic ring in the alkaline ethanolic medium. The corresponding aldehydes were reduced to the alcohols. It was, however, not possible to differentiate between the two compounds. Aldridge *et al.*[9] observed the formation of a lactol (14) besides (11) upon reduction of cytochalasin E with lithium aluminium hydride.

The cytochalasins are known for their specific effects on living cells.[10] The biological properties of cytochalasins E and K, as well as the reported derivatives are at present under investigation.

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus. U.v. absorptions were measured (for solutions in methanol) on a Unicam SP800 spectrometer, i.r. spectra on a Perkin-Elmer 237 spectrometer, optical rotations on a Perkin-Elmer 241 polarimeter, and mass spectra on a Varian-MAT 212 spectrometer. ^{1}H N.m.r. spectra were recorded in $CDCl_3$ on Varian EM-390 (90 MHz) and Varian XL-200 (200 MHz) spectrometers and ^{13}C n.m.r. spectra on a Varian CFT-20 spectrometer (20 MHz). C.d. spectra were obtained from a JASCO Model J-20 automatic spectropolarimeter. For column chromatography Merck silica, particle size 0.063—0.200 mm, or Merck silica gel H (type 60) under pressure (100 kPa) was used.

Isolation of Metabolites.—The isolate used was obtained from a sample of industrial sorghum malt and was deposited in the culture collection of the S.A. Medical Research Council as MRC 1181. *A. clavatus* MRC 1181 was grown in bulk on wet sterilized whole yellow maize for 21 days at 25 °C. The culture material was dried at 50 °C for 24 h, milled and 4.1 kg of this material was extracted with chloroform–methanol (1 : 1, v/v) for 48 h and the solvent evaporated under reduced pressure. The resultant crude extract was partitioned between 90% methanol (3 l) and hexane, and the methanol extract was evaporated to dryness. The residue was partitioned between chloroform and water and the chloroform extract evaporated to dryness to give a gum (21.0 g). This toxic material (20.0 g) was purified by chromatography on silica gel using benzene–acetone (4 : 1, v/v) to remove lipids. The remaining material was purified by column chromatography on silica gel under pressure (150 kPa) using chloroform–methanol (95 : 5, v/v) as eluant to give cytochalasins E (1) and K (2).

Cytochalasin E (1).—Cytochalasin E (1.1 g) had m.p. 210 °C (from acetone–n-hexane) (lit.,[2] 206—208 °C); $\lambda_{max.}$ (MeOH) 263, 257, and 252 nm (log ε 3.59, 3.78, and 3.73); $\nu_{max.}$ ($CHCl_3$) 3 410, 2 970, 1 762, 1 720, and 1 662 cm^{-1}; c.d. (MeOH) $\Delta\varepsilon_{325}$ 0, $\Delta\varepsilon_{294}$ −2.6, $\Delta\varepsilon_{259}$ −0.58, $\Delta\varepsilon_{229}$ −5.14, $\Delta\varepsilon_{218}$ 0; δ″ 7.40—7.00 (m, Ph), 6.45 (d, *J* 12 Hz, H-20), 6.10—5.70 (m, H-13), 5.90 (s, NH), 5.61 (d, *J* 12 Hz, H-19), 5.40—5.00 (m, H-14), 4.38 (s, OH), 3.80—3.60 (m, H-3), 3.10—2.50 (m, H-4,5, 7,8, 10, 16), 2.40—2.00 (m, H-15), 1.49 (s, 18-Me), 1.14 (s, H-12), 1.17 (d, *J* 5 Hz, 16-Me), and 1.10 (d, *J* 5 Hz, H-11), M^+ 495 (Found: C, 67.8; H, 6.85; N, 3.0%. $C_{28}H_{33}NO_7$ requires C, 67.86; H, 6.71; N, 2.83%).

Cytochalasin K (2).—Cytochalasin K (120 mg) had m.p. 246—248 °C (acetone–n-hexane); $\lambda_{max.}$ (MeOH) 263sh, 257, and 252sh nm (log ε 3.44, 3.64, and 3.58); $\nu_{max.}$ (KBr) 3 440, 2 920, 1 765, 1 720, and 1 662 cm^{-1}; c.d. (MeOH) $\Delta\varepsilon_{325}$ 0,

$\Delta\varepsilon_{290}$ −2.76, $\Delta\varepsilon_{260}$ −0.50, $\Delta\varepsilon_{239}$ −1.62, $\Delta\varepsilon_{229}$ 0, $\Delta\varepsilon_{221}$ 5.39; δ 7.26—7.04 (m, Ph), 6.57 (d, *J* 13 Hz, H-20), 6.21—6.05 (m, H-13), 5.70 (s, NH), 5.57 (d, *J* 13 Hz, H-19), 5.47—5.20 (m, H-14), 4.39 (s, OH), 3.87—3.75 (m, H-4,7), 3.38 (t, *J* 8 Hz, H-3), 3.00—2.50 (m, H-8, 10, 15a, 16), 2.08 (m, H-15b), 1.61 (s, Me), 1.47 (s, Me), 1.44 (s, Me), and 1.10 (d, *J* 6 Hz, 16-Me); M^+ 495 (Found: C, 67.2; H, 6.45; N, 2.65%. $C_{28}H_{33}NO_7$ requires C, 67.86; H, 6.71; N, 2.83%).

7-O-*Acetylcytochalasin K* (3).—Cytochalasin K (30 mg) in dry pyridine (1 ml) was treated with acetic anhydride (0.5 ml). After 1 h at 20 °C, standard work-up gave the *monoacetate* as a colourless solid (25 mg). It had $\nu_{max.}$ ($CHCl_3$) 3 420, 3 920, 1 765, 1 725, and 1 662 cm^{-1}; δ 7.40—7.10 (m, Ph), 6.62 (d, *J* 12 Hz, H-20), 6.24 (s, NH), 6.20—5.90 (m, H-13), 5.64 (d, *J* 12 Hz, H-19), 5.35 (d, *J* 9 Hz, H-7), 5.40—5.00 (m, H-14), 4.40 (s, OH), 3.90br (s, H-4), 3.50br (t, *J* 7 Hz, H-3), 3.10—2.70 (m, H-8, 10, 15a, 16), 2.05 (m, H-15b), 1.95 (s, OAc), 1.50 (s, 2 × Me), 1.48 (s, Me), and 1.13 (d, *J* 6 Hz, 16-Me); *m/e* 449 [10%, M^+ − (HOAc + CO)].

Oxidation of Cytochalasin K.—Chromium trioxide (40 mg) was added to pyridine (1 ml) and the resulting suspension was stirred with cytochalasin K (20 mg) for 1 h. The mixture was diluted with water and extracted with chloroform and the extract was washed with 1N-hydrochloric acid. Recovery of the product and purification by column chromatography ($CHCl_3$–methanol, 93 : 7) followed by recrystallisation from acetone–n-hexane yielded the product (4) as white needles, m.p. 210—211 °C. It had $\lambda_{max.}$ (MeOH) 246 (log ε 3.76); $\nu_{max.}$ (KBr) 3 420, 2 920, 1 765, 1 720, 1 680, and 1 660 cm^{-1}; *m/e* 493.208; $C_{28}H_{31}NO_7$ requires 493.210.

Acid Rearrangement of Cytochalasin E.—Cytochalasin E (100 mg) in THF (5 ml) was treated with 2N-H_2SO_4 (1 ml) and the mixture was heated under reflux for 30 min. After addition of water (50 ml), the solution was extracted with $CHCl_3$ (3 × 50 ml) and the combined organic extracts evaporated to dryness. Column chromatography (EtOAc-n-hexane, 7 : 3) of the residue yielded two compounds of which one (R_F 0.45) was identical with cytochalasin K (2). The other compound (R_F 0.31), 7,18-*dihydroxy*-16,18-*dimethyl*-10-*phenyl*-21,23-*dioxo*[13]-*cytochalasa*-6(12), 13(E), 19(E)*triene*-1,17,22-*trione* (5) crystallised from acetone–n-hexane as white crystals, m.p. 169—170 °C. It had $\nu_{max.}$ ($CHCl_3$) 3 420, 2 920, 1 765, 1 720, and 1 662 cm^{-1}; δ 7.40—7.10 (m, Ph), 6.56 (d, *J* 12 Hz, H-20), 6.30 (s, NH), 6.30—6.10 (m, H-13), 5.90—5.40 (m, H-14), 5.63 (d, *J* 12 Hz, H-19), 5.30 (s, H-12a), 5.16 (s, H-12b), 4.40 (s, OH), 3.83 (d, *J* 9 Hz, H-7), 3.30—2.50 (m, H-3, 4,5, 8, 10, 16), 2.20 (m, H-15), 1.50 (s, 18-Me), 1.16 (d, *J* 6 Hz, 11 or 16-Me), and 1.10 (d, *J* 6 Hz, 11 or 16-Me); M^+ 495 (Found: C, 67.7; H, 6.7; N 3.0. $C_{28}H_{33}NO_7$ requires C, 67.87; H, 6.66; N, 2.82%).

19,20-*Dihydrocytochalasin E* (9).—Cytochalasin E (30 mg) in EtOAc (10 ml) was stirred over Pd–C (30 mg) in a hydrogen atmosphere for 1 h at 20 °C. The catalyst was filtered off and the solvent evaporated under reduced pressure. Crystallisation of the residue from acetone–n-hexane yielded 19,20-*dihydrocytochalasin* E (9) as white needles (25 mg), m.p. 230 °C. It had $\nu_{max.}$ ($CHCl_3$) 3 410, 2 970, and 1 750 cm^{-1}; δ 7.40—7.10 (m, Ph), 6.60—6.30 (m, H-13), 6.25 (s, NH), 5.50—5.20 (m, H-14), 4.40 (s, OH), 4.50—4.20 (m, H-20), 3.70 (t, *J* 8 Hz, H-3), 3.30—2.60 (m, H-4, 5, 7, 8, 10, 16), 2.50—2.00 (m, H-15, 19), 1.46 (s, 18-Me), 1.16 (d, *J* 7 Hz, 16-Me), 1.13 (s, H-12), and 0.96 (d, *J* 7 Hz, H-11); *m/e* 337 (19%), 246 (17%), and 91 (80%) (Found: C, 67.35; H, 7.05; N 2.9. $C_{28}H_{35}NO_7$ requires C, 67.59; H, 7.09; N, 2.81%).

Methanolysis of Cytochalasin E.—Cytochalasin E (40 mg) was treated with 0.5N-aqueous $NaHCO_3$ (1 ml) and methanol (2 ml) at 20 °C (3 h). After addition of water (10 ml), the solution was extracted with $CHCl_3$ (3 × 10 ml). Evaporation of the combined organic extracts yielded the *aldehyde methyl ester* (10) as a colourless solid (25 mg). It had $\nu_{max.}$ ($CHCl_3$) 3 420, 2 920, 1 720, 1 440, and 1 270 cm^{-1}; δ 9.76 (s, CHO), 7.50—7.10 (m, Ph), 6.10—5.80 (m, H-10), 5.83 (s, NH), 5.70—5.30 (m, H-14), 4.10 (s, OH), 3.80 (s, OMe), 3.80—3.60 (m, H-3), 3.40—2.00 (m, H-4, 5, 7, 8, 10, 15, 16, 19), 1.31, 1.30 (s, 5, 16-Me), and 1.10 (d, *J* 6 Hz, 13,15-Me); *m/e* 440 [14%, M^+ − $C(CH_3)(OH)CH_2CHO$].

Sodium Borohydride Reduction of Cytochalasin E.—$NaBH_4$ (100 mg) was added to a solution of cytochalasin E (100 mg) in EtOH (10 ml) and the suspension was stirred for 1 h (20 °C). The solution was diluted with water, the ethanol was removed under reduced pressure, and the residue was acidified with hydrochloric acid. Extraction with chloroform gave a mixture of three products which was separated by column chromatography (chloroform–methanol 96 : 4 v/v). The major component (R_F 0.17), the 17-*hydroxy-derivative* (11), was obtained as a white solid (25 mg). It had $\nu_{max.}$ ($CHCl_3$) 1 765 and 1 720 cm^{-1}; δ 7.50—7.20 (m, Ph), 6.40 (d, *J* 12 Hz, H-20), 6.10—5.90 (m, H-13), 5.60 (d, *J* 12 Hz, H-19), 5.40—5.10 (m, H-14), 3.80—3.60 (m, H-3), 3.20—2.50 (m, H-4, 5, 7, 8, 10, 16), 2.20—2.00 (m, H-15), 1.30 (s, H-12, 18-Me), 1.20 (d, *J* 6 Hz, 16-Me), and 1.10 (d, *J* 6 Hz, H-11). The second compound (R_F 0.06) (15 mg) (12) or (13) had $\nu_{max.}$ ($CHCl_3$), 3 420, 2 920, 1 720, and 1 260 cm^{-1}; δ 7.40—7.10 (m, Ph), 5.90 (s, NH), 5.60—5.59 (m, H-13, 14), 4.20 (q, *J* 6 Hz, OCH_2CH_3), 4.00—3.00 (m, H-20), 3.20—1.80 (m, H-3, 4, 5, 7, 8, 10, 15, 16, 17, 19), 1.36 (s, H-12, 18-Me), 1.31 (t, *J* 6 Hz, OCH_2CH_3), 1.20 (d, *J* 6 Hz, 10-Me), and 1.10 (d, *J* 6 Hz, H-11); *m/e* 456 (7%, M^+−CO_2Et), 366 (100), and 310 (10).

The third compound (R_F 0.04) (15 mg) (12) or (13) had $\nu_{max.}$ ($CHCl_3$) 3 420, 2 920, 1 720, and 1 260 cm^{-1}; δ 7.50—7.20 (m, Ph), 5.90 (s, NH), 5.90—5.80 (m, H-13, 14), 4.21 (q, *J* 6 Hz, OCH_2CH_3), 4.00—3.60 (m, H-20), 3.40 (m, H-3), 3.20—2.00 (H-4, 5, 7, 8, 10, 15, 16, 17, 19), 1.31 (t, *J* 6 Hz, OCH_2CH_3), 1.30 (s, H-12), 1.26 (s, 8-Me), 1.15 (d, *J* 6 Hz, 16-Me), and 1.05 (d, *J* 6 Hz, H-11); *m/e* 456 (10%, M^+ −CO_2Et), 366 (100), and 310 (6).

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