The Structures of Cytochalasins A and B

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The structures of cytochalasins A and B, metabolites of *Helminthosporium dematioideum* showing novel effects on mammalian cells, have been determined. Cytochalasin B is identical with phomin. (−)-α-Methyladipic acid is shown to have the *R*-configuration contrary to a previous assignment; the absolute configurations of the *Nupheus* alkaloids therefore require revision.

OUR interest in the fungus *Helminthosporium dematioideum* arose from the observation [1] that the aqueous media on which it had grown produced unusual morphological effects on the hyphae of other fungi. A follow-up of this observation led to the isolation of two related compounds, one of which was responsible for the effects. Subsequently, both compounds were found [2] to produce novel effects on mammalian cells in tissue culture and the same effects were observed to be produced by filtrates from fermentations of other fungi, from one of which, *Metarrhizium anisopliae*, we have isolated a second pair of biologically active compounds related structurally to those from *H. dematioideum*. The name "cytochalasins" (*cytos* = cell, *chalasis* = relaxation) has been proposed for this class of compounds, those from *H. dematioideum* being cytochalasin A, which is responsible for the effects on fungal hyphae, and cytochalasin B; those from *M. anisopliae* are cytochalasins C and D.* This report describes work, a brief account of which has appeared,[3] which permits us to assign structures (Ia) and (Ib), respectively, to cytochalasins A and B.

The relationship (αβ-unsaturated ketone and allylic alcohol) between cytochalasin A, $C_{29}H_{35}NO_5$, and cytochalasin B, $C_{29}H_{37}NO_5$, could be deduced from their n.m.r. spectra (Table 1). In that of cytochalasin A, 2-H and 3-H give rise to an AB quartet, while in cytochalasin B 3-H is further coupled ($J = 6$ c./sec.) to a proton (4-H), not present in cytochalasin A, whose signal appears at τ 5·52 (C*H*–O). The relationship was confirmed by oxidation of cytochalasin B to cytochalasin A with manganese dioxide. The *trans*-configuration of the 2,3-double bond follows from the value of $J_{2,3}$ and is supported by the fact that irradiation of cytochalasin B with ultraviolet light causes isomerisation of the double bond to the *cis*-configuration, with the expected fall in value of $J_{2,3}$ to 12 c./sec.

Reduction of cytochalasin B with an excess of sodium borohydride gives a dihydro-compound (II) in which the 2,3-double bond has been reduced (no AB quartet in the n.m.r. spectrum of (II), signal due to 4-H shifted upfield to τ 6·2). Alkaline hydrolysis of the dihydro-compound gives the hydroxy-acid (IIIa) which on treatment with acid gives the γ-lactone (IVa) ($\nu_{max.}$ 1772 cm.$^{-1}$), isomeric with the dihydro-compound. This reaction sequence shows that, (i) the 2,3-double bond in cytochalasins A and B is conjugated to a lactone carbonyl group, (ii) the lactone ring must be large since the 2,3-double bond is *trans*-oriented, and (iii) the hydroxy-group involved in closure of the large lactone ring is tertiary since no new signals attributable to C*H*OH appear in the n.m.r. spectrum of the γ-lactone (IVa), though two signals, one a doublet and the other a sharp singlet, due to hydroxy-groups are apparent. The tertiary hydroxy-group in (IVa) or its derivatives is not acetylated by acetic anhydride in pyridine.

Cytochalasin A contains a secondary hydroxy-group [one-proton doublet (13-H) at τ 6·16] and forms a crystalline monoacetate; cytochalasin B also contains this group and forms an amorphous diacetate.

Their n.m.r. spectra show cytochalasins A and B to contain a second *trans*-disubstituted double bond, and double irradiation of cytochalasin A shows the double bond to be part of the system ·C(9)H(9′)H·C(10)H=C(11)H·C(12)H· ($J_{10,12}$ and $J_{9,11} = 0$) where τ 12-H is 7·05. Irradiation at τ 7·05 also causes the doublet due to 13-H to collapse to a broad singlet, suggesting that 12-H might be coupled to 13-H.† This was confirmed by oxidation of cytochalasin A to a diketone (Ie) in which 12-H is further deshielded and gives rise to a sharp doublet at τ 6·08; irradiation of this signal caused the signal due to 11-H to collapse to a double doublet.

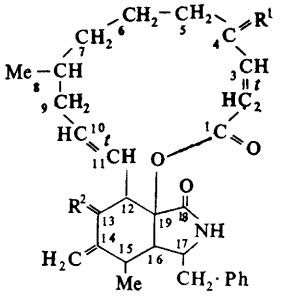
We have thus shown cytochalasins A and B to contain part structure (V) and this can be extended to (VI) on the following grounds. The n.m.r. spectra (Table 1) of cytochalasins A and B and many of their derivatives contain two broad singlets attributable to a methylene group the presence of which was confirmed by the formation of formaldehyde on ozonolysis of cytochalasin B, the dihydro-compound (II), or the γ-lactone (IVa). Hydrogenation of the γ-lactone over palladium leads to the formation of three products the molecular formulae and n.m.r. spectra (Table 1) of which are consistent with the structures (VII) (signal due to =C·CH_3 at τ 8·34; no signal due to ·C*H*OH), (VIII) (=C·CH_3 at 8·14; signal due to 13-H is a doublet), and (IX) [three signals due to ·CH·CH_3; 13-H gives rise to a double doublet and is shielded relative to 13-H in (VIII)]. Compound (IX) is more conveniently prepared by hydrogenation of the γ-lactone over platinum in methanol. Distribution between part-structure (VI) and the alternative in which

* Cytochalasins A and B are the subject of U.K. Patent Appln. No. 1,050,511; cytochalasins C and D are the subject of U.K. Patent Appln. No. 4182/66.

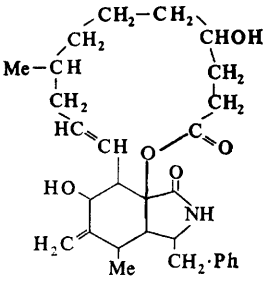
† The possibility of a second proton, with the same chemical shift as 12-H, coupled to 13-H cannot be excluded at this stage.

[1] H. G. Hemming, personal communication.
[2] S. B. Carter, *Nature*, 1967, **213**, 261.
[3] D. C. Aldridge, J. J. Armstrong, R. N. Speake, and W. B. Turner, *Chem. Comm.*, 1967, 26.

C-14 and C-15 are interchanged was made by oxidising the ketone (Xa) (τ 7·8, CH_3CO·), obtained by alkaline hydrolysis (reverse aldol reaction) of cytochalasin A,

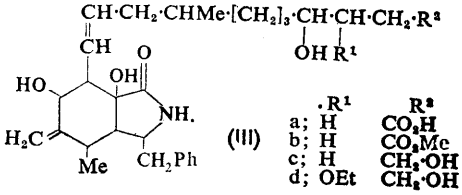


(I)



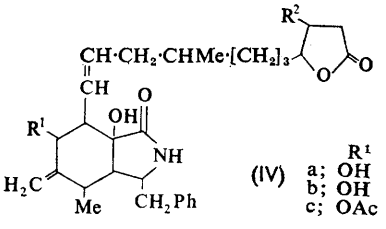
(II)

	R^1	R^2
a;	O	H,OH
b;	H,OH	H,OH
c;	O	H,OAc
d;	H,OAc	H,OAc
e;	O	O



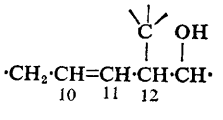
(III)

	R^1	R^2
a;	H	CO_2H
b;	H	CO_2Me
c;	H	CH_2OH
d;	OEt	CH_2OH

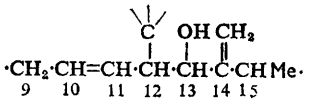


(IV)

	R^1	R^2
a;	OH	H
b;	OH	OEt
c;	OAc	OEt



(V)

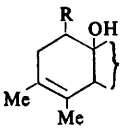


(VI)

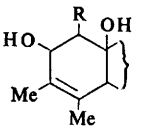
to the diketone (Xc); subtraction of the ultraviolet spectrum of (Xa) from that of (Xc) gave a difference curve ($\lambda_{max.}$ 230 mμ, ε 6200) characteristic of an αβ-unsaturated ketone, consistent with part-structure (VI).

The presence of a secondary methyl group in part-structure (VI) accounts for one of two such groups present in cytochalasin A [doublets at 8·92 and 9·07 shown by double irradiation to be coupled to protons at τ 6·9 (15-H) and 8·32 (8-H), respectively]. The second methyl group was shown to be attached to a methylene chain by the formation of (+)-β-methylpimelic acid [along with (+)-β-methyladipic, (−)-α-methyladipic, (−)-α-methylglutaric, methylsuccinic, succinic, and oxalic acids] on vigorous oxidation of cytochalasin B with concentrated nitric acid.

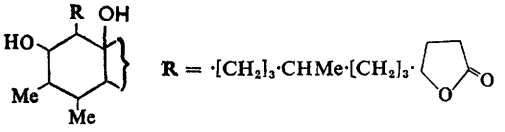
The inclusion of the methylene chain in the large-ring lactone was strongly indicated and was confirmed by ozonolysis of the γ-lactone (IVa) to give, as well as formaldehyde, the aldehyde (XIa) [$\nu_{max.}$ 2728 and 1727 (CHO), 1781 $cm.^{-1}$ (γ-lactone); τ 0·23t (CH_2·C*H*O), 5·55m (C*H*·O·CO), 9·05d (·CH·CH_3)] which was rapidly oxidised by air or by Jones reagent to the acid (XIb)



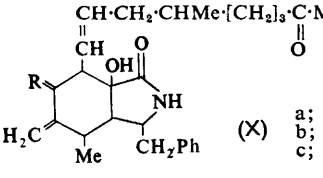
(VII)



(VIII)

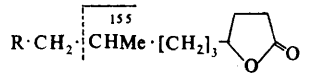


(IX)



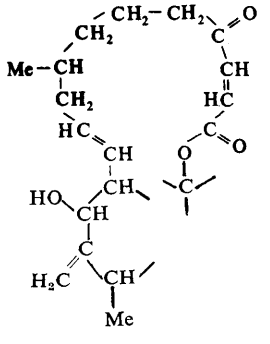
(X)

a;	R = H,OH
b;	R = H,OAc
c;	R = O

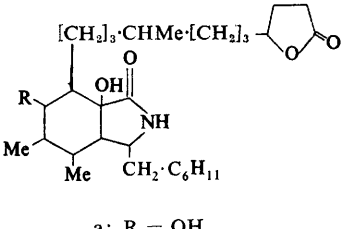


(XI)

a;	R = CHO
b;	R = CO_2H
c;	R = CO_2Me
d;	R = CONHPh

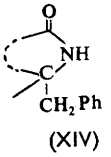


(XII)



(XIII)

a;	R = OH
b;	R = H



(XIV)

characterised as its methyl ester (XIc) and its anilide (XId). The position of the methyl group was revealed by the presence of a strong peak at *m/e* 155 in the mass

spectra of compounds (XI) due to the fragmentation shown.

Combining the evidence so far discussed we arrive at part-structure (XII) for cytochalasin A, leaving $C_{10}H_{10}NO$ unaccounted for. The presence of a phenyl group, probably as part of a benzyl group, in the cytochalasins and their derivatives is shown by their n.m.r. spectra (five-proton multiplet at about τ 2·8), their mass spectra (Table 3) (strong *m/e* 91, tropylium ion), and by infrared spectra. Thus, we arrive at a second part-structure (XIV) for cytochalasins A and B, leaving CH_2 unaccounted for and requiring the presence of a carbon ring.

Further information on the structure of cytochalasins A and B, and in particular on the nature of the lactam ring, was obtained by fusion of the γ-lactone (IVa) at 300° with a mixture of sodium and potassium hydroxides to give a 10% yield of the phthalimidine (XVa),

TABLE 1

Chemical shifts (τ values) for protons in cytochalasins A and B derivatives [a,b]

Compound	2-H	3-H	4-H	10-H	11-H	13-H	=CH_2	15-CH_2	8-CH_3	Miscellaneous
(Ia)	3·64d (16)	2·68d (16)		4·56m (15,9,3)	4·06m (15,9,2)	6·16d (11)	4·62, 4·88	8·92d (6)	9·07d (6)	
(Ib) [e]	4·19d (16)	3·06dd (16,6)	5·52m	4·62m (15,9,3)	4·02dd (15,8)	6·14d (11)	4·63, 4·86	9·00d (6)	9·14d (6)	
(Ie)	3·63d (16)	2·66d (16)		4·64m (17,9,4)	4·02m (17,10,2)		4·34, 4·66	8·82d (6)	9·08d (6)	12-H, 6·08d (10)
(II)			6·2m	~4·5m		6·2d [c] (~10)	4·56, 4·90	8·85d (6)	9·10d (6)	
(IVa)			5·60m	~4·4m	~4·8m	6·00d [d] (6)	4·84, 4·99	8·82d (6)	9·23d (6)	Sharp O*H* signal, 6·17
(VII)			5·60m					8·34s [f]	9·20d (6)	
(VIII)			5·58m			6·23d (14)		8·14s [f]	9·20d (6)	
(IX)			5·58m			6·90dd (14·3)		8·89d (6)	9·19d (6)	14-CH_3, 8·97d (6)
(XIIIa)			5·58m			~6·6m [c]		8·94d (6)	9·17d (6)	H_{17}, ~6·6m [c]; 14-CH_3 9·13d (6)
(XIIIb) (β-isomer)			5·58m					~9·05d	9·18d (6)	H_{17}, 6·5m; 14-CH_3 ~9·05d
(XIIIb) (γ-isomer)			5·58m					*f*	9·16d (6)	H_{17}, 6·8m
(XXIV)			~6·3m			~6·3m		8·31s	9·19d (6)	14-CH_3, 8·31s

[a] For conditions see Experimental section; figures in parentheses are coupling constants (c./sec.). [b] s = Singlet, d = doublet, dd = double doublet, m = multiplet. [c] These signals overlap. [d] Further coupled (J = 5) to a hydroxy proton (6·94d). [e] Measured in deuteriochloroform–trifluoroacetic acid. [f] The signals due to 14- and 15-CH_3 are not resolved.

TABLE 2

Nuclear magnetic resonance spectra (τ values) of phthalimidines (XV), (XVI), and (XXV) [a]

Compound	Ph	Ar·*H*	=C·H	H_A	H_M	H_X	Ar·CH_3
(XVa)	2·80m	3·05s (In benzene)		5·37dd (10,3) 5·58dd (8,3)	6·54dd (13,3) 6·78dd (14,3)	~8·5dd 7·50dd (14,8)	8·44, 8·72 (6H) 7·12, 7·90, 8·01
(XVb)		3·07s		5·43dd (10,2)	*b*	*b*	8·40, 8·75, 8·84
(XVIa)	3·76m	2·98s	3·43s				8·40, 8·52, 8·74
(XVIb)		3·05s	4·55d				8·38, 8·64, 8·68
(XXV) [e]	2·80m	3·01s		5·355dd (3,10)	6·53dd (3,14)	*d*	7·77 (6H)

[a] For explanation see Table 1. [b] The signals due to H_M and H_X are masked by the cyclohexyl protons. [c] The following signals are also present: 5·58m (C*H*·O·CO), 7·0t (4) (Ar·CH_2CH_2·), 9·17d (6) (·CH·CH_3). [d] The H_X signal is masked by other signals.

the formation of benzoic acid on vigorous oxidation of cytochalasin B. That the benzyl group is part of a phenethylamine system is indicated by the presence of an ion $C_8H_{10}N^+$ (*m/e* 120) in their mass spectra (Table 3), replaced by $C_8H_{16}N^+$ (*m/e* 126) in compounds [*e.g.*, (XIIIa), obtained by hydrogenation of the γ-lactone (IVa) over platinum in acetic acid] in which the phenyl ring has been reduced. The (neutral) nitrogen atom can be allocated to a lactam ring on the basis of, (i) the lack of fragmentation of the compounds on acid hydrolysis, (ii) the presence of a signal (N*H*) at about τ 4, due to a readily exchangeable proton, in their n.m.r. spectra, and (iii) the presence of a band at about 1695 cm.$^{-1}$ [*e.g.*, 1696 cm.$^{-1}$ in γ-lactone (IVa)] in their $C_{18}H_{19}NO$ ($\nu_{max.}$ 3185, 1688, and 1679 cm.$^{-1}$; $\lambda_{max.}$ 242, 282, and 290 mμ). The n.m.r. spectrum (Table 2) of the phthalimidine shows signals due to a phenyl ring, an isolated aromatic proton, an NH group, three aromatic methyl groups, and an AMX system. The mass spectrum of the phthalimidine shows a very strong $(M - 91)^+$ peak due to loss of benzyl (tropylium) ion. On catalytic hydrogenation (platinum), the phenyl ring is reduced to give the cyclohexyl compound (XVb) ($\nu_{max.}$ 3180, 1689, and 1682 cm.$^{-1}$; $\lambda_{max.}$ 243, 280·5, and 290 mμ) which shows a very strong $(M - 97)^+$ peak in the mass spectrum. In the n.m.r. spectrum (Table 2) of (XVb) the signals due to H_A and H_M are masked by those of the cyclohexyl protons but the signals due to H_X, the

aromatic proton, and the three aromatic methyl groups are still apparent.

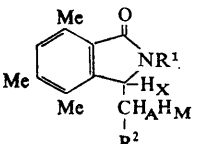
Dehydrogenation of the phthalimidine (XVa) over palladium gives the ethylenic compound (XVIa) ($\nu_{max.}$ 3230 and 1680 cm.$^{-1}$; $\lambda_{max.}$ 226, 231, 295, and 346 mμ; n.m.r. spectrum, Table 2) the mass spectrum of which contains no major peak. The corresponding hexahydro-compound (XVIb) ($\nu_{max.}$ 3170 and 1683 cm.$^{-1}$; $\lambda_{max.}$ 225·5, 232, 268, 320, and 334 mμ; n.m.r. spectrum, Table 2) may be obtained by oxidation of the hexahydrophthalimidine (XVb) with potassium permanganate.

The properties of the alkali fusion product define its structure as (XVa) or (XVII) and we hoped to distinguish between these two possibilities by synthesis of (XVII) which at the time seemed the more likely structure from consideration of possible biosynthetic routes to the corresponding structures, (I) and (XVIII), for the cytochalasins. However, our attempts, which will be described elsewhere, to synthesise (XVII) unambiguously were unsuccessful so we adopted an alternative approach to the determination of the orientation of the substituents in the alkali degradation product. The phthalimidine was converted with nitrous acid to the *N*-nitroso-compound (XVc) which on treatment with dilute alkali gave the corresponding phthalide (XIX).[4] Reduction of the phthalide with lithium aluminium hydride gave the diol (XXa) which was deoxygenated by the method of Cope [5] to give the hydrocarbon (XXc), m. p. 96—97°, the structure of which was proved by its synthesis from 1,2,4,5-tetramethylbenzene. The hydrocarbon (XXIb) derived from the alternative structure (XVII) for the degradation product was also synthesised (from 1,2,3,5-tetramethylbenzene) and had m. p. 35°. The structure of the phthalimidine was thus firmly established as (XVa) and of cytochalasins A and B as (Ia) and (Ib), respectively. The phthalimidine is optically inactive, presumably owing to racemisation during its formation.

During the course of this work Professor Tamm, of Basel University, informed us that cytochalasin B, the molecular formula of which had been published,[6] was isomeric with a compound, phomin, which he had isolated from a *Phoma* sp. and to which he had assigned structure (Ib). Comparison of cytochalasin B with phomin showed them to be identical. Professor Tamm's evidence for structure (Ib) [7]* is almost completely independent of our own; in particular he was able to obtain compounds (XXIIa) and (XXIIb) by ozonolysis of cytochalasin B.

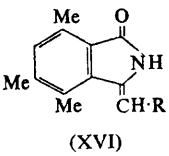
Several other reactions in the series may be interpreted in terms of structures (Ia) and (Ib). Hydrolysis of cytochalasin B in aqueous ethanol leads to the addition of ethanol across the 2,3-double bond to give the ethoxy-compound (IVb) which was reduced with lithium aluminium hydride to the tetrol (IIId) which, as expected, did not consume periodate. Reduction of dihydrocytochalasin B (II) or the γ-lactone (IVa) with lithium aluminium hydride gives the tetrol (IIIc). Reduction of the hydrogenolysis product (VII) gives the triol (XXIV).

We have already mentioned that reduction of the γ-lactone (IVa) with platinum in acetic acid gives the decahydro-γ-lactone (XIIIa). Similar reduction of the



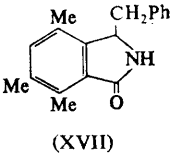
(XV)

a; R^1 = H, R^2 = Ph
b; R^1 = H, R^2 = C_6H_{11}
c; R^1 = NO, R^2 = Ph

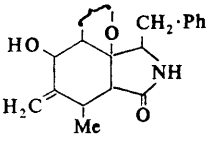


(XVI)

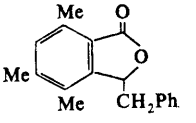
a; R = Ph
b; R = C_6H_{11}



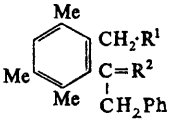
(XVII)



(XVIII)

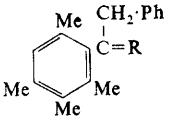


(XIX)



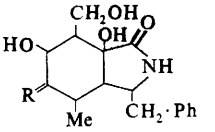
(XX)

a; R^1 = OH, R^2 = H,OH
b; R^1 = H, R^2 = O
c; R^1 = H, R^2 = H,H



(XXI)

a; R = O
b; R = H,H



(XXII)

a; R = H,OH
b; R = CH_2

hydrogenolysis product (VII) leads to the formation of three isomeric compounds, designated α-, β-, and γ-, which are presumably stereoisomers of structure (XIIIb); the α-compound is only formed in small amount.

Dehydrogenation of the hydrogenolysis product (VII) gives the phthalimidine (XXV) ($\nu_{max.}$ 1773 and 1688 cm.$^{-1}$; $\lambda_{max.}$ 282 and 291 mμ; n.m.r. spectrum, Table 2).

A major problem in the elucidation of the structures of cytochalasins A and B, was the determination of the nature and environment of the lactam ring, a problem which was finally solved by the alkali fusion experiment described above. The difficulty arises from the complex nature of the n.m.r. spectra, in which the signal due to 16-H is never visible and that due to 17-H is only clearly resolved in highly reduced compounds because one of the benzyl protons, which are usually magnetically non-

* We are indebted to Professor Tamm for permitting us to see his manuscript before publication.

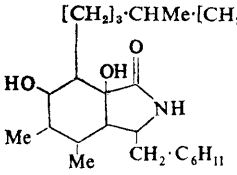
[4] C. Graebe, *Annalen*, 1888, **247**, 288.

[5] A. C. Cope, R. K. Bly, E. P. Burrows, O. J. Ceder, E. Ciganek, B. T. Gillis, R. F. Porter, and H. E. Johnson, *J. Amer. Chem. Soc.*, 1962, **84**, 2170.

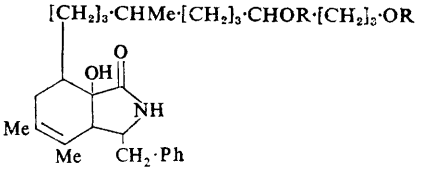
[6] D. C. Aldridge, J. J. Armstrong, R. N. Speake, and W. B. Turner, *4th International Symposium on the Chemistry of Natural Products*, Stockholm, 1966, Abstr., p. 109.

[7] W. Rothweiber and Ch. Tamm, *Experientia*, 1966, **22**, 750.

equivalent, has a chemical shift close to that of 17-H.* For example, in the spectrum of compound (IX) there are signals due to three protons—13-H, 19-H and one of

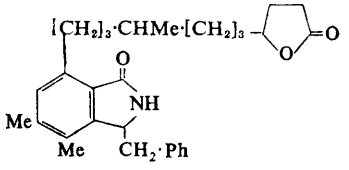


(XXIII)

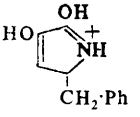


(XXIV)

a; R = H
b; R = Ac



(XXV)



(XXVI)

the benzyl protons—in the τ 6—7 region, while in the corresponding compound (XIIIa) in which the phenyl ring has been reduced the signals due to 13-H and 17-H overlap to form a complex multiplet. In the spectrum of the hydrogenolysis product (VII), the signals due to 17-H and one of the benzyl protons coincide; and while the signal due to 17-H is resolved from other signals in the isomeric compounds (XIIIb), it forms a complex pattern from which no clearcut information could be derived.

The mass spectra (Table 3) of compounds in the series show, in addition to the ions already discussed, a strong peak at *m/e* 190 corresponding to $C_{11}H_{12}NO_2^+$, replaced by a peak at *m/e* 196 ($C_{11}H_{18}NO_2^+$) in compounds in which the aromatic ring has been reduced. This ion, which must contain the elements of the lactam ring and the benzyl group, presumably has structure (XXVI) or one of its tautomers and serves to confirm the proximity of the tertiary hydroxy-group and the lactam ring.

The configurations of (—)-α-methylglutaric acid [8] and (+)-β-methyladipic acid [9] have been correlated with that of *R*-(+)-methylsuccinic acid and their formation in the nitric acid oxidation of cytochalasin B serves to define the configuration at C-8 as *R*. (+)-β-Methylpimelic acid had been previously synthesised [10] and could be allocated the *R*-configuration [7] on the basis of its derivation from (+)-pulegone. Since we also obtain (—)-α-methyladipic acid from the oxidation this, too, must have the *R*-configuration. However, (—)-α-methyladipic acid has been assigned the *S*-configuration [11] on the basis of its synthesis from (—)-α-methyl-γ-butyrolactone which was correlated with *S*-(—)-methylsuccinic acid. Comparison of the properties (m. p. 53—54°, $[\alpha]_D$ —1·4°) of the synthetic material with those (m. p. 79—80°, $[\alpha]_D$ —18°) of the compound derived from cytochalasin B shows that the former was not optically pure. (—)-α-Methyladipic acid, m. p. 82—83°, $[\alpha]_D$ —13°, has also been obtained by degradation of the alkaloid deoxynupharidine the absolute configuration of which was assigned [12] on the basis of (—)-α-methyladipic acid having the *S*-configuration. Our results show that these assignments, and the absolute configurations of the related alkaloids nupharamine,[13] dehydrodeoxynupharidine,[14] and nuphamine,[15] require revision.

TABLE 3

Mass-spectroscopic data [a]

Compound	M^+	$(M-91)^+$	$(M-97)^+$	*m/e* 196	*m/e* 190	*m/e* 126	*m/e* 120	*m/e* 91
(Ia)	13	100			9		8	40
(Ib)	15	53			10		22	100
(Id)	23	23			10		29	100
(Ie)	3·6	28			34		15	100
(II)	11	10			37		31	100
(IIId)	3	— [b]			65		51	100
(IVa)	2·5	8			45		28	100
(IXb)	1·2	6			65		41	100
(VII)	13	45			47		82	100
(VIII)	1·5	38			23		54	100
(IX)	11	100			41		39	95
(Xa)	5·4	16			70		39	100
(X) [c]	3·6	96			56		16	80
(XIIIa)	10		30	100		70		
(XIIIb) [d]	8·2		13	100		12		
(XXIII)	1·2		11	100		56		
(XXIVa)	11	15			27		100	100

[a] Figures are abundances as percentages of base peak ignoring peaks below *m/e* 91. [b] Negligible. [c] Base peak is at *m/e* 419 $[(M-18)^+]$. [d] The α-, β-, and γ-isomers gave almost identical mass spectra.

EXPERIMENTAL

Unless otherwise stated, infrared spectra were determined for Nujol mulls, ultraviolet spectra were measured for methanol or ethanol solutions, and n.m.r. spectra were for deuteriochloroform solutions with tetramethylsilane as internal standard, measured either at 60 Mc./sec. (results quoted in Experimental section) or at 100 Mc./sec. (results quoted in the

* Rothweiler and Tamm [7] avoided these problems by study of the (simpler) n.m.r. spectra of their ozonolysis products (XXIIa) and (XXIIb).

[8] E. J. Eisenbraun and S. M. McElvain, *J. Amer. Chem. Soc.*, 1955, **77**, 3383.
[9] J. von Braun and F. Jostes, *Chem. Ber.*, 1926, **59**, 1091.
[10] M. Mousseron and J. Jullien, *Bull. Soc. chim. France*, 1947, 605.
[11] T. Kaneko, K. Wakabayashi, and H. Katsura, *Bull. Chem. Soc. Japan*, 1962, **35**, 1149.
[12] Y. Arata and T. Iwai, *Kanazawa Daigaku Yakugakuba Kenkyu Nempo*, 1962, **12**, 39.
[13] I. Kawasaki, S. Matsutani, and T. Kaneko, *Bull. Chem. Soc. Japan*, 1963, **36**, 1474.
[14] Y. Arata, *Chem. Pharm. Bull. (Tokyo)*, 1964, **12**, 1394; 1965, **13**, 907.
[15] Y. Arata and T. Ohashi, *Chem. Pharm. Bull. (Tokyo)*, 1965, **13**, 1247.

Tables); double resonance experiments were at 100 Mc./sec. In the Experimental section the parentheses after τ values contain number of protons, multiplicity of signal (see footnote to Table 1), and coupling constant (J) in c./sec. Mass spectrometric data were determined in an AEI MS 9 spectrometer. Silica gel used for chromatography was either B.D.H. chromatographic grade or Hopkin and Williams M.F.C., and light petroleum had b. p. 60—80°. Thin-layer chromatography was on Merck silica gel G; preparative thin-layer chromatography was on layers 1 mm. thick and 20 cm. square with a maximum loading of 40—50 mg. of mixture per plate. When handling cytochalasin A and derivatives which contain the αβ-unsaturated ketone system, brown glassware was used to minimise isomerisation of the double bond.

Isolation and Characterisation of Cytochalasins A and B.—*Helminthosporium dematioideum* (CMI 74812; no. 1338 in our collection) was grown in Glaxo vessels each containing 250 ml. of Raulin Thom medium with 2·5% sugar. After 13 days the filtrate (33 l.) was extracted with chloroform (2 × 3 l.) and the solvent was removed to give a white solid (6·04 g.) which was absorbed on to silica gel (30 g.) by evaporation of an acetone solution. The silica gel was placed at the top of a column of silica gel (130 g.) suspended in a mixture of benzene and chloroform (9 : 1). Elution of the column with this solvent and with benzene–chloroform (4 : 1) yielded a small amount of gum which was discarded. Elution with benzene–chloroform (1 : 1) gave a mixture (488 mg.) of gum and solid which was crystallised three times from acetone–light petroleum to give *cytochalasin A* (Ia) (205 mg.) as felted needles, m. p. 182—185° (Found: C, 72·85, 73·0; H, 7·3, 7·4; N, 3·0, 3·1%; M, 477. $C_{29}H_{35}NO_5$ requires C, 72·9; H, 7·4; N, 2·9%; M, 477), $\nu_{max.}$ 3350s, 3210m, 3140w, 1714vs, 1692s, and 1623w cm.$^{-1}$; ultraviolet end absorption, ε_{207} 23,700.

Further elution of the column with benzene–chloroform (1 : 1) and then with chloroform gave first mixtures, m. p. 170—210°, and then a colourless solid (2·69 g.) which was recrystallised from acetone to give *cytochalasin B* (Ib) (1·61 g.) as felted needles, m. p. 218—221° (Found: C, 72·75, 72·5; H, 7·9, 8·0; N, 3·0%; M, 479·2671. $C_{29}H_{37}NO_5$ requires C, 72·6; H, 7·8; N, 2·9%; M, 479·2669); $\nu_{max.}$ 3510w, 3380s, 3225w, 3155w, 1715vs, 1692vs, 1638w, and 1605w cm.$^{-1}$; ultraviolet end absorption, ε_{207} 23,500.

The relative amounts of the cytochalasins produced in the fermentations has varied from time to time. Normally cytochalasin B is the major component and it is often convenient to crystallise the crude mixture from acetone to obtain the bulk of the cytochalasin B and then to chromatograph the mother-liquor as described above. On other occasions cytochalasin A has been the only cytochalasin present in the crude extract, and direct crystallisation has sufficed for its purification.

Cytochalasin A forms a *monoacetate* (Ic), m. p. 176—181° (Found: C, 71·6, 71·7; H, 7·3, 7·4. $C_{31}H_{37}NO_6$ requires C, 71·65; H, 7·2%); $\nu_{max.}$ 3195s, 3095s, 1732vs, 1703vs, and 1623s cm.$^{-1}$. Cytochalasin B forms a glassy *diacetate* (Id) (Found: M, 563·2893. $C_{33}H_{41}NO_7$ requires M, 563·2883); $\nu_{max.}$ 3220m(br), 1742vs, 1725vs, 1711vs, 1648w, and 1603w cm.$^{-1}$; τ 7·90 (3,s) and 8·05 (3,s).

Conversion of Cytochalasin B to Cytochalasin A.—A solution of cytochalasin B (500 mg.) in chloroform (50 ml.) was stirred with manganese dioxide (2 g.) at room temperature for 70 hr. The manganese dioxide was filtered off, the the chloroform evaporated, and the residue chromatographed on silica gel (25 g.). Elution with benzene–chloroform (1 : 1) yielded a solid (378 mg.) which was recrystallised from acetone–light petroleum to give cytochalasin A, identified by m. p., mixed m. p., i.r. spectrum, and t.l.c.

Photoisomerisation of Cytochalasin A.—A solution of cytochalasin A (40 mg.) in ethanol (10 ml.) was exposed to ultraviolet light overnight at room temperature, the solution was concentrated to 1—2 ml., and the resulting crystals (23 mg.) were recrystallised from ethanol to give the 2,3-*cis-isomer*, m. p. 228—234° (Found: C, 72·9; H, 7·4; N, 3·0%); $\nu_{max.}$ 3350m, 3210m, 3140w, 1710vs, 1685vs, 1644w, 1632w, and 1607w cm.$^{-1}$; ultraviolet end absorption, ε_{200} 30,000; τ (in deuteriochloroform–trifluoroacetic acid) 3·45 (1, d, J = 12) and 3·6 (1, d, J = 12).

Preparation of the Ketone (Ie).—(*a*) 8N-Chromic acid (0·075 ml.) was added during 10 min. to a stirred suspension of cytochalasin A (100 mg.) in acetone (1·5 ml.) at 0°. The mixture was diluted with water and extracted with ethyl acetate to give a yellow gum (111 mg.) which was chromatographed on silica gel. Elution with benzene–chloroform (3 : 2) gave a solid (42 mg.) which was recrystallised from ether–light petroleum to give the *ketone* (Ie) as prisms, m. p. 166—174° (Found: C, 73·4; H, 6·8; N, 3·1%; M, 475. $C_{29}H_{33}NO_5$ requires C, 73·2; H, 7·0; N, 3·1%; M, 475); $\nu_{max.}$ 3210w, 3100w, 1718vs, 1699s, and 1625w cm.$^{-1}$.

(*b*) A suspension of cytochalasin B (500 mg.) in acetone (6 ml.) was treated with 8N-chromic acid (0·9 ml.) to give the ketone (Ie) (140 mg.).

Vigorous Oxidation of Cytochalasin B.—(a) *With potassium permanganate.* A mixture of cytochalasin B (135 mg.), sodium carbonate (225 mg.), water (10 ml.), and 1,2-dimethoxyethane (3 ml.) was heated under reflux until a clear solution was obtained. A portion of the solution was distilled to remove the dimethoxyethane, and water (10 ml.) was added followed by powdered potassium permanganate (360 mg.) in small portions. The permanganate was decolourised almost immediately. The mixture was heated under reflux for 1 hr. when further permanganate (500 mg.) was added and the heating continued for a further hour when the permanganate had again been consumed. A further quantity (380 mg.) of permanganate was added and the heating continued for a further 3 hr. The cooled mixture was filtered free from manganese dioxide, acidified with hydrochloric acid, and extracted with ether to give a semisolid product (57 mg.) which was purified by sublimation at 5 × 10^{-2} mm. and recrystallisation from water to give benzoic acid (14 mg.), m. p. 122—123°, identical with an authentic sample.

(b) *With nitric acid.* Cytochalasin B (3 g.) was treated with concentrated nitric acid (50 ml.) at room temperature for 1 hr. and then at the boiling point for 2·5 hr. Water (100 ml.) was added and a portion (50 ml.) of the mixture was distilled. Further water (50 ml.) was added and a further portion (50 ml.) was distilled. Extraction of the combined distillates with ether yielded no steam-volatile material. The residue from the distillation was then evaporated to dryness, treated with water (50 ml.) and again evaporated to dryness. This process was repeated twice and the residue was extracted with water (20 ml.) leaving a solid (250 mg.) which was sublimed to give *p*-nitrobenzoic acid, identified by comparison with an authentic sample. The aqueous solution was evaporated to dryness and the residue was extracted with ether and methylated with ethereal diazomethane, and the esters were separated by

v.p.c. in a Wilkens Autoprep A700 using a column (20 ft. × ⅜ in. outside diam.) packed with Chromasorb W (30—60 mesh) containing 20% butanediolsuccinate at 210° with a helium flow-rate of 120 ml./min. Seven fractions were obtained and were examined by 60 Mc. n.m.r. spectroscopy in carbon tetrachloride and then hydrolysed overnight at room temperature in a mixture of methanol (11·6 ml.) and 5N-sodium hydroxide (1·6 ml.), to give (fraction number in parentheses): (1) 9·1 mg., (2) 12·7 mg., (3) 5·8 mg., (4) 68·0 mg., (5) 47·1 mg., (6) 42·4 mg., and (7) 44·6 mg. Fractions (1) to (3) were shown by their spectra and those of their esters to be oxalic, methylsuccinic, and succinic acid, respectively. Fractions (4)—(7) were purified by chromatography on silica gel and elution with chloroform–ethyl acetate to give, respectively, (−)-α-methylglutaric acid, m. p. 79—81°, $[\alpha]_D^{28}$ −22·8° (*c* 0·45 in EtOH) (Found: C, 49·6; H, 6·9. Calc. for $C_6H_{10}O_4$: C, 49·3; H, 6·9%); (−)-α-methyladipic acid, m. p. 79—80°, $[\alpha]_D^{28}$ −18·0° (*c* 0·45 in EtOH) (Found: C, 52·9; H, 7·6. Calc. for $C_7H_{12}O_4$: C, 52·5; H, 7·6%); (+)-β-methyladipic acid, m. p. 86—88°, $[\alpha]_D^{28}$ +10·7° (*c* 0·25 in EtOH) (Found: C, 52·8; H, 7·7%); and (+)-β-methylpimelic acid as a gum, $[\alpha]_D^{25}$ +5·0° (*c* 0·7 in EtOH), characterised as its *bisanilide*, m. p. 157—158° (Found: C, 74·2; H, 7·4; N, 8·8%; *M*, 324. $C_{20}H_{24}N_2O_2$ requires C, 74·0; H, 7·5; N, 8·6%; *M*, 324).

Dihydrocytochalasin B (II).—Sodium borohydride (3 g.) was added over 0·5 hr. to a stirred solution of cytochalasin B (3 g.) in methanol (180 ml.) and the stirring was continued for a further hour. The mixture was diluted with water and the methanol was removed under reduced pressure. The residue was diluted with brine, acidified with hydrochloric acid, and extracted with chloroform to give a pale yellow foam (3·4 g.) which was chromatographed on silica gel. Elution with chloroform yielded fractions (2·6 g.) shown by t.l.c. to be a single compound. Recrystallisation from ethyl acetate yielded *dihydrocytochalasin B* (II) as needles, m. p. 198—203° (Found: C, 72·4; H, 8·4; N, 2·9%; *M*, 481. $C_{29}H_{39}NO_5$ requires C, 72·3; H, 8·2; N, 2·9%; *M*, 481); $\nu_{max.}$ 3460m, 3265w, 3150sh, 1736vs, 1710vs, 1642w, and 1603w cm.$^{-1}$; $\lambda_{max.}$ 253, 258·5, 264, and 268 mμ (ε 195, 224, 174, and 135).

Preparation of the Hydroxy-acid (IIIa).—A solution of dihydrocytochalasin B (44 mg.) in a mixture of ethanol (2·7 ml.) and 3N-sodium hydroxide (1·3 ml.) was heated under reflux for 1 hr. The solution was cooled, cautiously acidified with hydrochloric acid, and immediately extracted with ethyl acetate. Removal of the solvent and crystallisation of the product from acetone gave the *hydroxy-acid* (IIIa) (22 mg.) as microcrystals, m. p. 186—188° (Found: C, 69·7; H, 8·4; N, 3·0. $C_{29}H_{41}NO_6$ requires C, 69·7; H, 8·3; N, 2·8%); $\nu_{max.}$ 3330m(br), 3210sh, 2700br, 1710vs, 1693vs, 1643w, and 1605w cm.$^{-1}$.

The methyl ester (IIIb), prepared with diazomethane, formed needles, m. p. 129—130° (Found: C, 70·1; H, 8·4. $C_{30}H_{43}NO_6$ requires C, 70·15; H, 8·4%); $\nu_{max.}$ 3495w, 3220br, 1722s, 1691vs, 1642w, and 1605 cm.$^{-1}$.

Preparation of the γ-Lactone (IVa).—(*a*) A solution of the hydroxy-acid (IIIa) (11 mg.) and *NN'*-dicyclohexylcarbodi-imide (15 mg.) in 1,2-dimethoxyethane (3 ml.) was set aside at room temperature overnight. The solvent was evaporated and the residue was chromatographed on silica gel. Elution with chloroform yielded the *γ-lactone* (IVa) as felted needles (from ethyl acetate or acetone), m. p. 192—193° (Found: C, 72·6; H, 8·3; N, 2·9%; *M*, 481. $C_{29}H_{39}NO_5$ requires C, 72·3; H, 8·2; N, 2·9%; *M*, 481); $\nu_{max.}$ 3520w, 3320m, 3080w, 1772s, 1696vs, 1645w, and 1603w cm.$^{-1}$.

(*b*) The γ-lactone (IVa) is more conveniently prepared directly from dihydrocytochalasin B by warming the acidified hydrolysis mixture on a water-bath for 15 min. followed by extraction with ethyl acetate and recrystallisation.

Reduction of the γ-Lactone (IVa) *with Lithium Aluminium Hydride*.—Lithium aluminium hydride (31 mg.) was slowly added to a solution of the γ-lactone (31 mg.) in tetrahydrofuran (8 ml.) and the mixture was set aside at room temperature for 1 hr. and then heated under reflux for 2 hr. The mixture was acidified with 3N-sulphuric acid and extracted with ethyl acetate to give a gummy solid which was chromatographed on silica gel. Elution with ethyl acetate–methanol (96 : 4) gave a solid (20 mg.) which was recrystallised from acetone–light petroleum to give the *tetrol* (IIIc), m. p. 144—146° (Found: C, 71·4; H, 8·9; N, 2·9. $C_{29}H_{43}NO_5$ requires C, 71·7; H, 8·9; N, 2·9%); $\nu_{max.}$ 3250br, 1694s, 1644w, and 1603w cm.$^{-1}$.

Reduction of Dihydrocytochalasin B with Lithium Aluminium Hydride.—Dihydrocytochalasin B (274 mg.) was reduced as described above to give the tetrol (IIIc) (53 mg.).

Alkaline Hydrolysis of Cytochalasin A.—A solution of cytochalasin A (1 g.) in a mixture of ethanol (40 ml.) and 3N-sodium hydroxide (160 ml.) was heated under reflux in an atmosphere of nitrogen for 1 hr. The methanol was removed under reduced pressure and the solution was acidified with hydrochloric acid and extracted with ethyl acetate. The product was divided into a neutral fraction (374 mg.) and an acid fraction (640 mg.) with sodium hydrogen carbonate. The acid fraction was shown by t.l.c. to be a mixture of at least four components, which could not be separated by column chromatography. The neutral fraction, which was essentially a single compound, was chromatographed on silica gel. Elution with chloroform yielded a solid (269 mg.) which was recrystallised from acetone–light petroleum to give the *methyl-ketone* (Xa) as needles, m. p. 209—210° (Found: C, 74·0; H, 8·7; N, 3·2%; *M*, 439. $C_{27}H_{37}NO_4$ requires C, 73·8; H, 8·5; N, 3·2%; *M*, 439); $\nu_{max.}$ 3500m, 3226m, 1717s, 1698vs, 1650w, and 1609w cm.$^{-1}$; $\lambda_{max.}$ 253, 259, 265, and 268 mμ (ε 177, 200, 168, and 134); τ 7·8 (3, S).

The ketone (Xa) forms a *monoacetate* (Xb), needles, m. p. 202—204° (Found: C, 72·6; H, 8·0. $C_{29}H_{39}NO_5$ requires C, 72·3; H, 8·2%); $\nu_{max.}$ 3300m, 1740s, 1719s, and 1697s cm.$^{-1}$; τ 7·88 (3, s) and 8·00 (3, s).

Alkaline Hydrolysis of Cytochalasin B.—A solution of cytochalasin B (506 mg.) in a mixture of ethanol (30 ml.) and 3N-sodium hydroxide (15 ml.) was heated under reflux for 90 min. The ethanol was removed under reduced pressure, and the aqueous solution was acidified with hydrochloric acid and extracted with ethyl acetate. The product was chromatographed on silica gel. Elution with chloroform–ethyl acetate (5 : 1) gave a solid which was recrystallised from ethyl acetate–light petroleum to give the *ethoxy-γ-lactone* (IVb) (78 mg.) as needles, m. p. 161—164° (Found: C, 70·4; H, 8·2; N, 3·2%; *M*, 525. $C_{31}H_{43}NO_6$ requires C, 70·8; H, 8·2; N, 2·7%; *M*, 525); $\nu_{max.}$ 3520w, 3320m, 3200m, 1776s, 1694s, and 1600w cm.$^{-1}$.

The ethoxy-γ-lactone (IVb) forms an *acetate* (IVc) m. p. 194—195° (Found: C, 69·7; H, 7·8; N, 3·2. $C_{33}H_{45}NO_7$ requires C, 69·8; H, 8·0; N, 2·5%); $\nu_{max.}$ 3310m, 1777s, 1738s, and 1698vs cm.$^{-1}$. Elution of the silica

column with chloroform–ethyl acetate (1 : 1) yielded a solid mixture of acidic compounds, shown by t.l.c. to contain four components.

Reduction of the Ethoxy-γ-lactone (IVb) *with Lithium Aluminium Hydride*.—A solution of the ethoxy-γ-lactone (175 mg.) in dry tetrahydrofuran (10 ml.) was added to a suspension of lithium aluminium hydride (160 mg.) in tetrahydrofuran (5 ml.) and the mixture was set aside at room temperature for 45 min. The mixture was cautiously acidified with 3N-sulphuric acid and extracted with chloroform to give the product (116 mg.) which was crystallised from ethyl acetate–light petroleum to give the *ethoxy-tetrol* (IIId) as a hydrate, m. p. 115—118° (Found: C, 68·5; H, 8·8%; *M*, 529. $C_{31}H_{47}NO_6,H_2O$ requires C, 68·0; H, 9·0%. $C_{31}H_{47}NO_6$ requires *M*, 529); $\nu_{max.}$ 3500—3200br, 1680br, and 1600w cm.$^{-1}$. The tetrol did not consume periodate.

Oxidation of the Methyl Ketone (Xa).—8N-Chromic acid (0·1 ml.) was added portionwise to a stirred suspension of the methyl ketone (79 mg.) in acetone (2 ml.) at 0°. The mixture was diluted with water and extracted with ethyl acetate. The product was chromatographed on silica gel. Elution with chloroform–benzene (2 : 3) yielded a solid (40 mg.) which was recrystallised from acetone–light petroleum to give the *diketone* (Xc), m. p. 147—157° (Found: C, 74·3; H, 8·1; N, 2·9%; *M*, 437. $C_{27}H_{35}NO_4$ requires C, 74·1; H, 8·1; N, 3·2%; *M*, 437); $\nu_{max.}$ 3296m 3182m, 3107w, 1713s, 1698s, 1622m, and 1603w cm.$^{-1}$; λ_{infl} 230 mμ (ε 6600); τ 7·89 (3, s) and 6·5 (1, d, J = 8).

Hydrogenation of the γ-Lactone (IVa).—(a) *With palladised charcoal*. A solution of the γ-lactone (900 mg.) in methanol (50 ml.) was shaken with palladised charcoal (5% palladium; 500 mg.) in an atmosphere of hydrogen for 36 hr. The product was recovered and chromatographed on silica gel. Elution with chloroform yielded a solid (378 mg.) which was crystallised from ethyl acetate–light petroleum to give the *hydrogenolysis product* (VII) as needles, m. p. 168—172° (Found: C, 74·1; H, 8·9; N, 2·75%; *M*, 467. $C_{29}H_{41}NO_4$ requires C, 74·5; H, 8·8; N, 3·0%; *M*, 467); $\nu_{max.}$ 3340m, 3260sh, 1780s, 1692s, and 1606w cm.$^{-1}$.

Elution of the silica gel column with chloroform–ethyl acetate gave a gummy solid (585 mg.) shown by t.l.c. to contain the hydrogenolysis product (VII) along with two further compounds at lower R_F. The mixture was separated by t.l.c. in ethyl acetate, appropriate fractions being recovered with acetone. In this way were isolated (*a*) (lowest R_F) the *iso-dihydro-γ-lactone* (VIII) as a gum containing some tetrahydro-compound (Found: *M*, 483·2998. $C_{29}H_{41}NO_5$ requires *M*, 483·2985); $\nu_{max.}$ (film) 3300m(br), 1772s, 1700s, and 1606w cm.$^{-1}$; and (*b*) (intermediate R_F) the *tetrahydro-γ-lactone* (IX) as needles, m. p. 134—135° (Found: C, 71·4; H, 8·8; N, 2·7%; *M*, 485. $C_{29}H_{43}NO_5$ requires C, 71·7; H, 8·9; N, 2·9%; *M*, 485); $\nu_{max.}$ 3470m, 3385m, 3090br, 1773vs, and 1695vs cm.$^{-1}$.

(b) *With platinum in methanol*. A solution of the γ-lactone (150 mg.) in methanol (3 ml.) was shaken with hydrogen in the presence of platinum (from platinum oxide, 75 mg.) until rapid consumption of hydrogen had ceased. Removal of the catalyst and solvent gave a product shown by t.l.c. to contain the tetrahydro-γ-lactone (IX) as major component. Preparative t.l.c. in ethyl acetate followed by crystallisation of the appropriate fractions from ethyl acetate–light petroleum gave the pure tetrahydro-compound (59 mg.).

(c) *With platinum in acetic acid*. The γ-lactone (50 mg.) in acetic acid (5 ml.) was shaken with hydrogen in the presence of platinum (from platinum oxide, 50 mg.) for 1 hr. The product was freed from a minor gummy component (lower R_F) by t.l.c. in ethyl acetate to give the *decahydro-γ-lactone* (XIIIa) as needles (28 mg.), m. p. 132—134° (Found: C, 70·5; H, 9·7; N, 2·7%; *M*, 491. $C_{29}H_{49}NO_5$ requires C, 70·8; H, 10·0; N, 2·8%; *M*, 491), $\nu_{max.}$ 3480m, 3384m, 3100br, 1776s, and 1695s cm.$^{-1}$.

Hydrogenation of the Hydrogenolysis Product (VII).—A solution of the hydrogenolysis product (109 mg.) in acetic acid (5 ml.) was shaken with hydrogen in the presence of platinum (from platinum oxide, 100 mg.) for 2 hr. Recovery of the product and t.l.c. in ethyl acetate gave (i) (minor component, highest R_F) the *α-decahydrodesoxy-γ-lactone* (XIIIb), needles, m. p. 145—146° (Found: C, 72·8; H, 10·5; N, 2·8%; *M*, 475. $C_{29}H_{49}NO_4$ requires C, 73·2; H, 10·4; N, 2·9%; *M*, 475); $\nu_{max.}$ 3330m, 3260sh, 1785s, 1689s, and 1684s cm.$^{-1}$, (ii) (intermediate R_F) the *β-decahydrodesoxy-γ-lactone* (XIIIb), needles, m. p. 118—121° (Found: C, 73·1; H, 10·4; N, 2·8%; *M*, 475); $\nu_{max.}$ 3390m, 3246sh, 1783s, and 1691s cm.$^{-1}$, and (iii) (lowest R_F) the *γ-decahydrodesoxy-γ-lactone* (XIIIb), needles, m. p. 142—147° (Found: C, 73·1; H, 10·6; N, 2·9%; *M*, 475); $\nu_{max.}$ 3340m, 3280sh, 1783s, and 1690s cm.$^{-1}$.

Reduction of the Hydrogenolysis Product (VII) *with Lithium Aluminium Hydride*.—A solution of the hydrogenolysis product (100 mg.) in dry tetrahydrofuran (5 ml.) was added to a suspension of lithium aluminium hydride (200 mg.) in the same solvent (5 ml.) and the mixture was set aside at room temperature for 0·5 hr. 3N-Sulphuric acid was added cautiously with cooling and the product was extracted with chloroform to give a gummy solid (99 mg.) which was treated in ethyl acetate with a little charcoal and crystallised from ethyl acetate–light petroleum to give the *triol* (XXIVa) as hydrated needles, m. p. 124—133° (Found: C, 72·7, 72·5, 72·6; H, 9·4, 9·5, 9·4; N, 2·8, 2·6, 2·7%; *M*, 471. $C_{29}H_{45}NO_4,0·5H_2O$ requires C, 72·5; H, 9·6; N, 3·0%. $C_{29}H_{45}NO_4$ requires *M*, 471); $\nu_{max.}$ 3330s, 3240sh, 1692s, and 1605w cm.$^{-1}$.

The triol forms a *diacetate* (XXIVb), m. p. 118—120° (Found: C, 71·3; H, 9·1; N, 2·3. $C_{33}H_{49}NO_6$ requires C, 71·3; H, 8·9; N, 2·5%); $\nu_{max.}$ 3345m, 3240m, 1736s, 1692s, 1667m, and 1607w cm.$^{-1}$; τ 8·04s (6H).

Reduction of the Decahydro-γ-lactone (XIIIa) *with Lithium Aluminium Hydride*.—The decahydro-γ-lactone (28 mg.) was reduced as described in the previous experiment to give a gum which was chromatographed on silica gel. Elution with chloroform and with chloroform–acetone gave only traces of material. Elution with acetone gave a gum (18 mg.) which was crystallised from ethyl acetate–light petroleum to give the *tetrol* (XXIII) as needles, m. p. 108—112° (Found: C, 69·8; H, 10·4; N, 2·7%; *M*, 495. $C_{29}H_{50}NO_5$ requires C, 70·3; H, 10·8; N, 2·8%; *M*, 495); $\nu_{max.}$ 3350br and 1693s cm.$^{-1}$.

Ozonolysis of Cytochalasin B.—Ozonised oxygen (approximately 80 mg. ozone) was passed through a solution of cytochalasin B (200 mg.) in acetic acid (10 ml.) at room temperature. Zinc dust (200 mg.) and water (5 ml.) were added and the mixture was set aside at room temperature for 1 hr. The mixture was poured into brine and extracted with ethyl acetate to give a gum from which no useful product could be obtained. The aqueous layer was adjusted to pH 6—7 with sodium hydrogen carbonate solution, and a saturated aqueous solution (15 ml.) of dimedone was added

and the solution was set aside to crystallise. Recrystallisation of the product from ethanol gave formaldehyde dimethone (44 mg.), m. p. 188—190°, identical with an authentic sample.

Ozonolysis of Dihydrocytochalasin B.—Ozonised oxygen (15 mg. ozone/min.) was passed for 3 min. through a solution of dihydrocytochalasin B (60 mg.) in acetic acid (8 ml.). Zinc dust (120 mg.) and water (10 ml.) were added, and the mixture was set aside for 30 min. The mixture was distilled until 8 ml. had been collected and the distillate was added to a solution of 2,4-dinitrophenylhydrazine in 3N-hydrochloric acid. The resulting precipitate was recrystallised from ethanol–light petroleum to give formaldehyde 2,4-dinitrophenylhydrazone, m. p. 166—168°, identical with an authentic sample.

Ozonolysis of the γ-Lactone (IVa).—A stream of ozonised oxygen (15 mg. oxone/min.) was passed for 10 min. through a solution of the γ-lactone (335 mg.) in methylene dichloride (40 ml.) at −15°. The excess of ozone was removed with nitrogen and the solution was added dropwise to a suspension of zinc dust in hot water (100 ml.) so that the methylene dichloride evaporated. The cooled mixture was set aside for 0·5 hr. and extracted with ethyl acetate to give a yellow gum (299 mg.) which was chromatographed on silica gel (13·5 g.). Elution with benzene–chloroform (7 : 3) yielded fractions (85 mg.) containing oils shown by t.l.c. to be mixtures of two compounds. The major component was obtained pure by rechromatography on silica gel to give the aldehydo-lactone (XIa) as a colourless oil, $\nu_{max.}$ (film): 2728w, 1781s, and 1727s cm.$^{-1}$; τ 0·23 (1, t), 5·55 (1, m), and 9·05 (3, d). On exposure to air the aldehyde is rapidly oxidised to the corresponding acid (XIb), which may also be obtained by oxidation of the aldehyde with 8N-chromic acid. The acid forms a *methyl ester* (XIc) as an oil, b. p. 100—120° (bath)/0·4 mm. (Found: C, 63·0; H, 8·7. $C_{12}H_{20}O_4$ requires C, 63·1; H, 8·8%); $\nu_{max.}$ (film) 1782s and 1742s cm.$^{-1}$; and an *anilide* (XId), m. p. 74—76° (Found: C, 70·4; H, 8·1; N, 4·8. $C_{17}H_{23}NO_3$ requires C, 70·6; H, 8·0; N, 4·8%); $\nu_{max.}$ 3244m, 3196m, 3135w, 3058w, 1775vs, 1659vs, 1600s, and 1548s cm.$^{-1}$.

Continued elution of the first silica gel column with chloroform–benzene and with chloroform gave gums shown by t.l.c. chromatography to be mixtures which could not be separated.

Selenium Dehydrogenation of the Hydrogenolysis Product (VII).—A finely divided mixture of the hydrogenolysis product (206 mg.) and selenium (1·5 g.) was heated at 300° for 1 hr. in an atmosphere of nitrogen. The mixture was then extracted with hot chloroform to give a yellow gum (205 mg.) which was chromatographed on silica gel (10 g.). Elution with benzene–chloroform (1 : 1) yielded a gum (81·5 mg.) shown by t.l.c. to be mainly one compound which was obtained pure by t.l.c. in chloroform–acetone (9 : 1). The *phthalimidine* (XXV) (42 mg.) was thus obtained as a colourless gum (Found: *M*, 447·2763. $C_{29}H_{37}NO_3$ requires *M*, 447·2773); $\nu_{max.}$ (film) 3190m, 1773s, 1688s, and 1593w cm.$^{-1}$; $\lambda_{max.}$ 282 and 291 mμ (ε 1690 and 1720); λ_{infl} 243 mμ (ε 7660); n.m.r. spectrum, Table 2.

Alkali Fusion of the γ-Lactone (IVa).—The finely divided γ-lactone (1·5 g.) was intimately mixed in a nickel crucible with a powdered mixture of potassium hydroxide (10 g.) and sodium hydroxide (10 g.) and the mixture was placed for 25 min. in a furnace at 300° through which nitrogen was flowing. The cooled mixture was extracted with water and the aqueous suspension was extracted with chloroform to give a yellow gum (1·08 g.) which was chromatographed on silica gel (20 ml.). Elution with benzene yielded a brown gum (384 mg.) and elution with benzene–chloroform (7 : 3) yielded a pale yellow solid (330 mg.) which was crystallised from acetone–light petroleum to give *3-benzyl-4,5,7-trimethylphthalimidine* (XVa) as prisms, m. p. 187—189°, $[\alpha]_D^{25}$ 0° (*c* 0·5 in EtOH) (Found: C, 81·2; H, 6·9; N, 5·1%; *M*, 265. $C_{18}H_{19}NO$ requires C, 81·5; H, 7·2; N, 5·3%; *M*, 265); $\nu_{max.}$ 3185m, 3075m, 1688s, 1679s, and 1600m cm.$^{-1}$; $\lambda_{max.}$ 242, 282, and 290 mμ (ε 8200, 1540, and 1700).

3-Cyclohexylmethyl-4,5,7-trimethylphthalimidine (XVb).—A solution of 3-benzyl-4,5,7-trimethylphthalimidine (32 mg.) in glacial acetic acid (4 ml.) was shaken with hydrogen and platinum (from platinum oxide, 20 mg.) for 1 hr. The catalyst was removed and the product crystallised from acetone to give *3-cyclohexylmethyl-4,5,7-trimethylphthalimidine* (XVb) as needles, m. p. 223—224° (Found: C, 80·0; H, 9·4; N, 5·0%; *M*, 271. $C_{18}H_{25}NO$ requires C, 79·7; H, 9·3; N, 5·2%; *M*, 271); $\nu_{max.}$ 3180m, 3080m, 1689s, 1682s, and 1596m cm.$^{-1}$; $\lambda_{max.}$ 243, 280·5, and 290 mμ (ε 11,920, 2927, and 3171).

3-Benzylidene-4,5,7-trimethylphthalimidine (XVIa).—A mixture of 3-benzyl-4,5,7-trimethylphthalimidine (30 mg.) and palladium black (100 mg.) were heated at 260° for 2 hr. and then the pressure was reduced to 0·1 mm. until sublimation was complete. The product (higher R_F) was separated from residual starting material by t.l.c. in methanol–chloroform (5 : 95) and was recrystallised from acetone to give *3-benzylidene-4,5,7-trimethylphthalimidine* (XVIa) as plates, m. p. 232—233° (Found: C, 82·2; H, 6·6; N, 5·3%; *M*, 263. $C_{18}H_{17}NO$ requires C, 82·1; H, 6·5; N, 5·3%; *M*, 263); $\nu_{max.}$ 3230m, 1680s, 1642m, 1605w, and 1580w cm.$^{-1}$; $\lambda_{max.}$ 226, 231, 295, and 346 mμ (ε 22,540, 22,540, 13,820, and 19,730).

3-Cyclohexylmethinyl-4,5,7-trimethylphthalimidine (XVIb).—A solution of the cyclohexyl compound (XVIa) (41 mg.) in acetone (15 ml.) was stirred with potassium permanganate (200 mg.) for 16 hr. The excess permanganate was reduced with methanol, the mixture evaporated to dryness, and the residue re-suspended in water and extracted with chloroform to give a solid (15·7 mg.) which was purified by sublimation at 220°/0·3 mm. and crystallisation from acetone to give *3-cyclohexylmethinyl-4,5,7-trimethylphthalimidine* (XVIb) as plates, m. p. 286—287·5° (Found: C, 80·2; H, 8·63; N, 4·8%; *M*, 269. $C_{18}H_{23}NO$ requires C, 80·3; H, 8·6; N, 5·2%; *M*, 269); $\nu_{max.}$ 3170m, 3040w, 1683s, and 1590w cm.$^{-1}$; $\lambda_{max.}$ 225·5, 232, 268, 320, and 334 mμ (ε 26,820, 27,520, 18,200, 11,590, and 11,300).

3-Benzyl-4,5,7-trimethyl-2-nitrosophthalimidine (XVc).—Sodium nitrite (230 mg.) was added to a solution of 3-benzyl-4,5,7-trimethylphthalimidine (230 mg.) in a mixture of formic acid (90%; 20 ml.) and water (5 ml.) and the mixture was set aside for 15 min. during which time a yellow solid separated. Further sodium nitrite (230 mg.) was added and the mixture was set aside for a further 15 min. and extracted with chloroform to give a yellow solid which was chromatographed on silica gel (1·5 × 9 cm.). Elution with benzene yielded a yellow solid (239 mg.) which was crystallised from acetone–light petroleum to give *3-benzyl-4,5,7-trimethyl-2-nitrosophthalimidine* (XVc) as yellow prisms, m. p. 156—157° (Found: C, 74·0; H, 6·1; N, 9·1. $C_{18}H_{18}N_2O_2$ requires C, 73·5; H, 6·2; N, 9·5%); $\nu_{max.}$ 1747s, 1688m, 1611m, and 1587m cm.$^{-1}$; τ 3·0 (4, m), 3·55 (2, m), 4·51 (1, dd, *J* = 3, 4), 6·6 (1, dd, *J* = 4, 14), 6·9 (1, dd, *J* = 3, 14), 7·51 (3, s), 7·61 (3, s), and 7·68 (3, s).

3-Benzyl-4,5,7-trimethylphthalide (XIX).— N-Sodium hydroxide (5 ml.) was added to a solution of the nitroso-compound (174 mg.) in dioxan (10 ml.) and the solution was set aside for 30 min. during which time the yellow colour was discharged with evolution of gas. The solution was acidified with concentrated hydrochloric acid, set aside for a further 30 min., diluted with water and extracted with chloroform to give a solid (160 mg.) which was crystallised from acetone–light petroleum to give *3-benzyl-4,5,7-trimethylphthalide* (XIX) as prisms, m. p. 129—130° (Found: C, 80·9; H, 6·7. $C_{18}H_{18}O_2$ requires C, 81·2; H, 6·8%); $\nu_{max.}$ 1755s, 1619w, and 1593w cm.$^{-1}$; τ 2·73 (5, m), 3·03 (1, s), 4·61 (1, dd, J = 5·5, 8), 5·0 (1, d, J = 12), 5·44 (1, d, J = 12), 6·9 (2, m), 7·67 (3, s), 7·77 (3, s), and 7·79 (3, s).

1-(2-Hydroxymethyl-3,5,6-trimethylphenyl)-2-phenylethanol (XXa).—A suspension of lithium aluminium hydride (100 mg.) in tetrahydrofuran (2 ml.) was added to a solution of the phthalide (XIX) (106 mg.) in tetrahydrofuran (5 ml.) and the mixture was set aside at room temperature for 30 min. The excess hydride was cautiously decomposed with water and the mixture was acidified with hydrochloric acid and extracted with chloroform to give a gum which was crystallised from acetone–light petroleum to give *1-(2-hydroxymethyl-3,5,6-trimethylphenyl)-2-phenylethanol* (XXa) as prisms, m. p. (double) 78—79° and 99—100° (Found: C, 79·7; H, 8·1. $C_{18}H_{22}O_2$ requires C, 80·0; H, 8·2%); $\nu_{max.}$ 3577s, 3402s, 1603w, and 1584w cm.$^{-1}$.

1,2,4,5-Tetramethyl-3-phenethylbenzene (XXc).— (*a*) Benzyl 2,3,5,6-tetramethylphenyl ketone (XXb) [16] (100 mg.) was heated under reflux overnight with zinc amalgam [from zinc dust (2·5 g.), and mercuric chloride (125 mg.)] and 6N-hydrochloric acid. The cooled mixture was extracted with ether to give a solid (96 mg.) which was chromatographed on silica gel (1·3 × 7 cm.). Elution with light petroleum gave a solid (79 mg.) which was crystallised from aqueous ethanol to give *1,2,4,5-tetramethyl-3-phenethylbenzene* (XXc) as plates, m. p. 97—98° (Found: C, 90·9; H, 9·2%; M, 238. $C_{18}H_{22}$ requires C, 90·7; H, 9·3%; M, 238); $\nu_{max.}$ 1599w, 862m, 743s, 732w, and 690s cm.$^{-1}$; τ (carbon tetrachloride) 2·87 (5, m), 3·31 (1, s), 7·25 (4, m), and 7·83 (9, s).

(*b*) A mixture of the diol (XXa) (86 mg.), red phosphorus (120 mg.), and hydriodic acid (3 ml.) was stirred and heated under reflux for 18 hr. The mixture was cooled, diluted with water, and extracted with chloroform. The chloroform extract was washed with 2% sodium thiosulphate solution, dried, and evaporated to give a gummy solid (76 mg.) which was dissolved in tetrahydrofuran and heated under reflux with lithium aluminium hydride (100 mg.) for 2 hr. The reaction was worked-up to give a gum (58 mg.) which was chromatographed in benzene–light petroleum (1 : 4) on a layer of silica gel. Elution of the highest R_F band (blue fluorescence in u.v. light) gave a solid (27 mg.) which was recrystallised from aqueous ethanol to give the hydrocarbon (XXc), plates, m. p. 97—99°, identical with the synthetic material.

1,3,4,5-Tetramethyl-2-phenethylbenzene (XXIb).—Benzyl 2,3,4,6-tetramethylphenyl ketone (XXIa) [17] was reduced as described for ketone (XXb) to give *1,3,4,5-tetramethyl-2-phenethylbenzene* (XXIb) as needles, m. p. 35—35·5° (Found: C, 90·6; H, 9·1%; M, 238); $\nu_{max.}$ 1604w, 1584w, 870m, 751s, 700s, and 692 cm.$^{-1}$; τ (in carbon tetrachloride) 2·87 (5, m), 3·31 (1, s), 7·25 (4, m), 7·81 (6, s), and 7·87 (3, s).

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