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ISOLATION OF BREFELDIN A FROM *PHYLLOSTICTA MEDICAGINIS**

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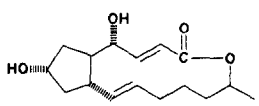
Abstract—Cultivation of the fungus, *Phyllosticta medicaginis*, afforded good yields of brefeldin A, mannitol, and fatty-acid glycerides as the principle metabolites The fatty-acid content of the glycerides was determined

INTRODUCTION

LITTLE work has been carried out on the fungal genus, *Phyllosticta* During initial investigations into the metabolites of this species, we have investigated *Phyllosticta medicaginis* and isolated a compound which has been isolated from other fungi under a variety of names As in other previous work on *Penicillium brefeldianum*,[1] we use the name brefeldin A for this compound, it has also been isolated from *Penicillium cyaneum* as cyanein, *Penicillium decumbens* as decumbin,[3,4] and *Ascochyta imperfecta* as ascotoxin [4]

RESULTS AND DISCUSSION

The mother liquors from the cultivation of *Phyllosticta medicaginis* were extracted with ethyl acetate to yield only *one* detectable metabolite (**1**) on TLC (2 g from 6 l of culture medium Compound (**1**), $C_{16}H_{24}O_4$, was shown by its physical and chemical properties to be brefeldin A



(1)

The "pelt" of fungus on the culture medium was dried and extracted to yield mannitol and a mixture of fatty-acid glycerides These glycerides were saponified and the free fatty-acids were esterified with diazomethane The composition of the fatty acid mixture was investigated by GC–MS before and after catalytic hydrogenation, and is reported in Table 1 Bu'lock has postulated a polyunsaturated fatty acid precursor reacting with oxygen to give brefeldin,

* Part II in the series "Fungal Metabolites" For Part I see *J Chem Soc Perkin I*, in press

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similar to the proposed mechanism of formation of prostaglandins from arachidonic acid [5] Although the conversion of fatty acid to prostaglandins or brefeldin probably takes place entirely on an enzyme(s) without the intermediates becoming free [6] we investigated the fatty acid composition in *Phyllosticta medicaginis* after 10 and 20 days growth to see which acids were present As shown in Table 1 the composition of the fatty acid mixture is comparable with that found in other investigations [7] of fungi of the *Curvularia* genera from which curvularin and brefeldin A have been isolated Only the presence of a C_{16} monoethenoic acid seems at all exceptional

TABLE 1 PERCENTAGES OF TOTAL FATTY ACID ISOLATED FROM THE LIPID FRACTION OF *Phyllosticta medicaginis**

Growth time (Days)	Palmitic	C_{16} Monoethenoic	Stearic	Oleic	Linoleic	Linolenic
10	25 2	4 1	3 0	34 8	32 1	1 7
20	32 5	3 3	4 3	40 9	16 8	2 0

* A very small quantity of C_{14} acid has been neglected

EXPERIMENTAL

Fungal culture Phyllosticta medicaginis (obtained from Centraalbureau voor Schimmelcultures Baarn The Netherlands) was grown for 20 days at 25 in static culture on the following medium potato extract (0 5% w/v Difco) dextrose (1 5% w/v) in dist H_2O

Brefeldin A The fungal "pelt" was filtered off and the aqueous filtrate was extracted 3 × with EtOAc (0 5 vol of each portion of the filtrate) The combined extracts were dried ($MgSO_4$) and evaporated to yield brefeldin A as a pale-yellow solid which crystallized from MeOH H_2O in colourless needles m p 205–206 $[\alpha]_D^{23}$ +93 5° (*C* 0 32 MeOH) λ_{max}^{EtOH} 227 nm (log ε 4 6) ν_{max}^{KBr} 3360 (–OH) 1708 (α,β-unsaturated carbonyl) 1640 cm^{-1} (>C=C<) δ (DMSO-d_6) 1 15 (3H *d*, *J* 6Hz >CH–CH$_3$), 5 66 (1H *q* *J* 16 *J* 2Hz –CO–CH CH–CHOH) 7 29 (1H *q* *J* 16 and *J* 3Hz, –CO–CH=CH=CHOH) (Found C, 68 5 H 8 6 Calc for $C_{16}H_{24}O_4$ C 68 6 8 6%)

Mannitol and glycerides The fungal pelt was dried at 60° for 2 days crushed and extracted continuously with EtOAc for 2 days After concentration of the extract mannitol separated as an off-white solid which crystallized from EtOH as colourless needles m p 166–167 , ν_{max}^{KBr} 3300 cm^{-1} With Ac_2O the hexaacetate was formed m p 124 (colourless needles from EtOH H_2O) (Found C 49 6 H 5 8 Calc for $C_{18}H_{26}O_{12}$ C 49 8 6 0%) After separation of the mannitol, the concentrated extract was further evaporated to a viscous brown oil which was extracted with 40–60 light petrol to yield a mixture of glycerides The glycerides were refluxed in methanolic potassium hydroxide for 1 hr to give the free fatty acids which were methylated with diazomethane The mixture of methyl esters was investigated by GC MS on a 1 5 m column of celite coated with 10% EGSS X (Applied Science Labs) at 180 and a gas flow of 45 ml min

Derivatives of Brefeldin A On hydrogenation over 10% Pd-C at atmospheric pressure tetrahydrobrefeldin A was obtained [2] m p 133 (diethyl ether) (Found C 67 4 H, 9 7 Calc for $C_{16}H_{28}O_4$ C 67 5 H 9 9%), $[\alpha]_D^{22}$ +3 1 (*c* 1 0 MeOH) With acetic anhydride pyridine Brefeldin A gave a diacetate [2] m p 132 5 133 5 (MeOH–H_2O), $[\alpha]_D^{25}$ +15 (*c* 0 80 MeOH) δ $CDCl_3$ 1 23 (3H *d* *J* 6Hz >CHCH$_3$), 2 00 (3H s) and 2 08 (3H s, –COCH$_3$) 5 18 (1H *q* *J* 14 9Hz >CH–CH=CH–) 5 68 (1H *q* *J* 14 6Hz –CH$_2$–CH=CH–) 5 68 (1H *q* *J* 16 2Hz –CHOH–CH=CH–CO–) 7 20 (1H *q* *J* 16 3Hz –CHOH–CH=CH–CO–) (Found C, 65 5 H 7 6 Calc for $C_{20}H_{28}O_6$ C, 65 9, H, 7 7%) The diacetate (40 mg) in dry Et_2O (10 ml) was added to Li (15 mg) in liq NH_3 (20 ml) and left for 30 min at −30 The NH_3 was then allowed to evaporate H_2O was added and neutralized with 3 N HCl, and the whole was extracted with Et_2O to give an oil (14 mg dehydroxybrefeldin A) which failed to crystallize, M W 264 (MS), $\nu_{max}^{CHCl_3}$ cm^{-1} 3450 (OH) 1710 (γ,β-unsaturated carbonyl) When treated with *m*-chloroperbenzoic acid in $CHCl_3$ at room temp for 24 hr brefeldin yielded the 10 11-monoepoxide [5] m p 219 221 (MeOH) $[\alpha]_D^{25}$ −22 9 (*C* 0 25 pyridine) (Found C 65 0 H 8 3 Calc for $C_{16}H_{24}O_5$ C 64 9 H 8 2%)

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