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Cyclographin, a New Depsidone from the Lichen *Catarraphia dictyoplaca*

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Abstract

The new depsidone cyclographin (1) (methyl 2-chloro-4-formyl-3,7-dihydroxy-1,6,9-trimethyl-11-oxo-11*H*-dibenzo[*b*,*e*][1,4]dioxepin-8-carboxylate) has been isolated from the lichen *Catarraphia dictyoplaca* and the structure established by spectroscopic and degradative methods.

Introduction

During a chemotaxonomic survey of representative lichens from the Pacific Islands it became apparent that the lichen *Catarraphia dictyoplaca* (Mont. & van den Bosch) Massal. (= *Cyclographa interposita* Vainio)[1] was chemically unique, producing a relatively non-polar compound of unknown constitution. We have now isolated this compound and have shown it to be the new depsidone cyclographin (1) by spectroscopic and degradative means.

The New Depsidone

Cyclographin (1) was the major component isolated from the acetone extract of the lichen *Catarraphia dictyoplaca* and was purified by preparative thin layer chromatography. The mass and ^{1}H n.m.r. spectra of this compound confirmed that it was isomeric with the well known depsidone physciosporin (2).[2–4] High resolution mass measurement on the molecular ion established that the molecular formula of (1) was $C_{19}H_{15}ClO_8$. The ^{1}H n.m.r. spectrum of cyclographin (1) exhibited three *C*-methyl resonances at δ 2·59, 2·45 and 2·27, a singlet at 3·98 due to an *O*-methyl group, two downfield singlets at 12·92 and 11·26 due to the intramolecularly hydrogen-bonded hydroxy groups, and a singlet at 10·91 attributed to an aldehyde proton.

Chromatographic (t.l.c) comparisons of cyclographin (1) and physciosporin (2) established that they were non-identical. Thus, assuming a normal biogenesis of this depsidone,[5,6] the most likely structure for cyclographin would appear to be

[1] Egea, J. M., and Torrente, P., *Crytogamie, Bryol. Lichénol.*, 1993, **14**, 329.
[2] Maass, W. S. G., McInnes, A. G., Smith, D. G., and Taylor, A., *Can. J. Chem.*, 1977, **55**, 2839.
[3] Goh, E. M., and Wilkins, A. L., *J. Chem. Soc., Perkin. Trans. 1*, 1979, 1656.
[4] Elix, J. A., and Lajide, L., *Aust. J. Chem.*, 1981, **34**, 2005.
[5] Elix, J. A., Whitton, A. A., and Sargent, M. V., *Fortschr. Chem. Org. Naturst.*, 1984, **45**, 104.
[6] Elix, J. A., Jenie, U. A., and Parker, J. L., *Aust. J. Chem.*, 1987, **40**, 1451.

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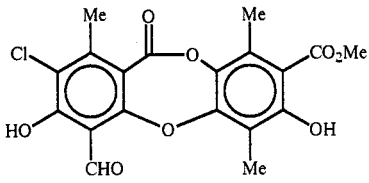
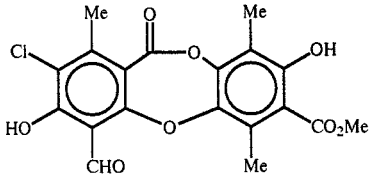
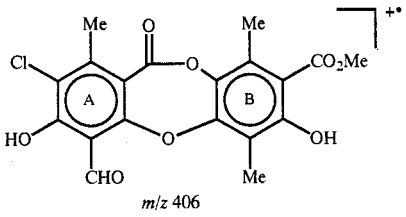
(1). Such an A-ring substitution pattern was supported by the mass spectrum of cyclographin, which exhibited significant daughter ion peaks at *m/z* 211/213 and 213/215 (Scheme 1), this clearly accounting for the expected functional groups. Further the observed loss of 32 mass units from the molecular ion of cyclographin (1) indicated the presence of a methoxycarbonyl substituent adjacent to a free hydroxy and/or methyl group.



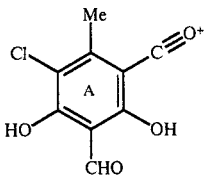
(1)



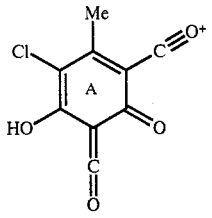
(2)



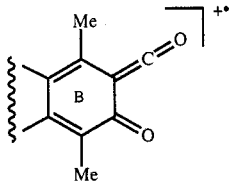
m/z 406



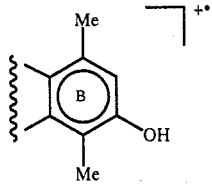
(*a*) *m/z* 213/215
$C_9H_6ClO_4$



(*b*) *m/z* 211/213
$C_9H_4ClO_4$



m/z 374



m/z 346

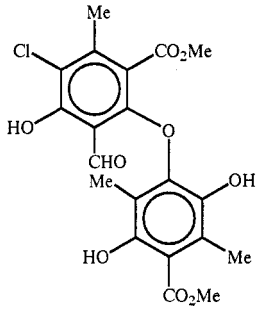
Scheme 1

Degradation Products of Cyclographin (1) and Physciosporin (2)

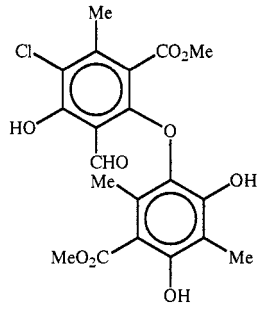
Cleavage of the depsidone ester linkage of cyclographin (1) by acid-catalysed methanolysis afforded a phenolic diaryl ether, subsequently shown to be methyl 5-chloro-2-(2′,5′-dihydroxy-4′-methoxycarbonyl-3′,6′-dimethylphenoxy)-3-formyl-4-hydroxy-6-methylbenzoate (3), in reasonable yield (62%). The ^1H

n.m.r. spectrum of this degradation product was highly informative; it showed the presence of three *C*-methyl resonances (all singlets) at δ 2·43, 2·34 and 2·18, two methoxy resonances at 3·91 and 3·50, two low field hydroxy singlets at 12·60 and 11·32 and a formyl resonance at 10·42. The mass spectrum of (3) further showed a molecular ion peak at m/z 438 and the expected fragment ions.

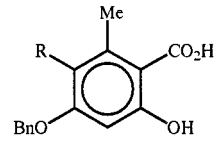
Methanolysis (by base or acid catalysis) of the corresponding depsidone ester linkage of physciosporin (3) afforded methyl 5-chloro-2-(2′,4′-dihydroxy-5′-methoxycarbonyl-3′,6′-dimethylphenoxy)-3-formyl-4-hydroxy-6-methylbenzoate (4) identical in all respect to authentic material.[2] The ^{1}H n.m.r. and mass spectrum of (4) were analogous, but not identical, to those of the isomer (3). In turn this confirmed that cyclographin (1) was an isomer of physciosporin (2), and that the total synthesis of the degradation product (3) would constitute a proof of structure of the precursor depsidone (1). A synthetic approach to (3) was undertaken starting from the appropriately substituted A- and B-ring precursors.



(3)



(4)



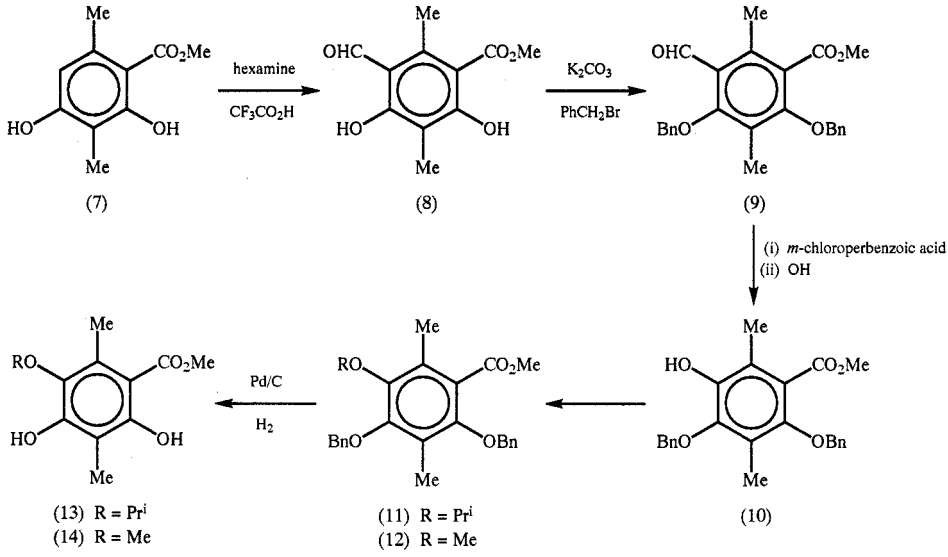
(5) R = H
(6) R = Cl

Total Synthesis of a Diaryl Ether (3)

In the initial approach the mononuclear A-ring precursor (6) was prepared by chlorination of 4-benzyloxy-2-hydroxy-6-methylbenzoic acid (5) with sulfuryl chloride. The preparation of an appropriate B-ring precursor (13) was a more difficult task and the synthetic route to this compound is outlined in Scheme 2. Formylation of methyl 2,4-dihydroxy-3,6-dimethylbenzoate (7) was achieved by treatment with hexamine and trifluoroacetic acid (the modified Duff method) to afford the 3-formyl compound (8).[7] *O*-Benzylation of (8) with benzyl bromide and anhydrous potassium carbonate afforded the protected aldehyde, methyl 4,6-dibenzyloxy-3-formyl-2,5-dimethylbenzoate (9). Oxidation of this aldehyde (9) by treatment with *m*-chloroperoxybenzoic acid followed by hydrolysis of the intermediate formate afforded the phenol (10). Alkylation of (10) with 2-bromopropane and anhydrous potassium carbonate gave the 5-(propan-2′-yloxy) derivative (11). Subsequent hydrogenolysis of this compound yielded methyl 2,4-dihydroxy-3,6-dimethyl-5-(propan-2′-yloxy)benzoate (13). The condensation of (6) and (13) could not be effected by treatment with trifluoroacetic anhydride but succeeded smoothly in the presence of *N*,*N*-dicyclohexylcarbodiimide to give the depside ester (15), together with trace amounts of the isomer (16) and

[7] Pulgarin, C., Gunzinger, J., and Tabacchi, R., *Helv. Chim. Acta*, 1985, **68**, 1948.

4-benzyloxy-3-chloro-6-hydroxy-2-methylbenzoic anhydride. The depside ester (15), was expected to afford the diaryl ether (18) upon Smiles rearrangement. However, when (15) was treated with potassium carbonate in anhydrous dimethyl sulfoxide, no reaction was observed. Steric crowding by the 5-(propan-2′-yloxy) substituent may well be responsible for inhibiting this reaction.



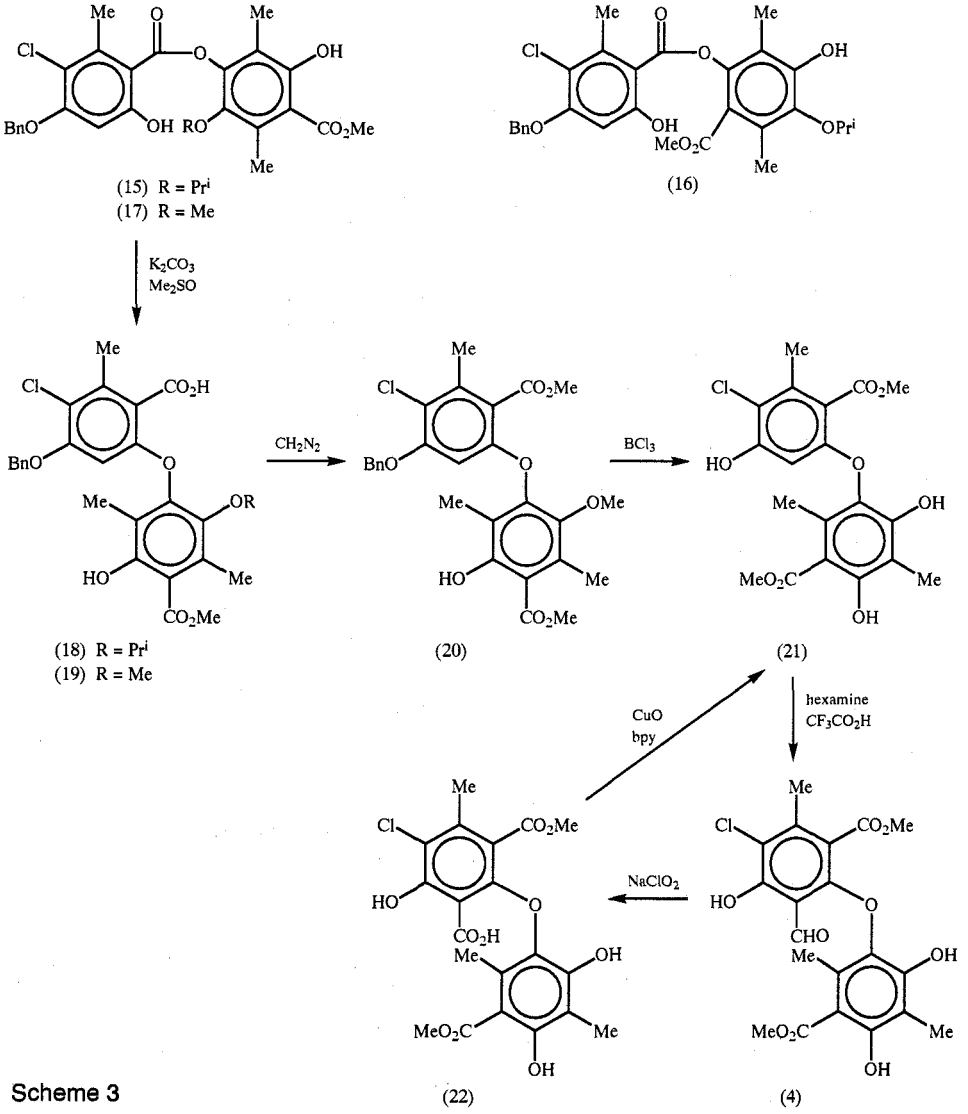
Scheme 2

As a result, an alternative less bulky B-ring precursor was sought. Hence *O*-methylation of the phenol (10) gave methyl 2,4-dibenzyloxy-5-methoxy-3,6-dimethylbenzoate (12) and subsequent hydrogenolysis of (12) afforded methyl 2,4-dihydroxy-5-methoxy-3,6-dimethylbenzoate (14).

Condensation of the acid (6) with the phenol (14) was effected by treatment with *N*,*N*-dicyclohexylcarbodiimide to afford the depside ester (17). The structure of the depside ester (17) followed from the microanalytical and the spectroscopic evidence. In particular the ^{1}H n.m.r. spectrum of (17) revealed a singlet due to the aromatic proton (H 5′) at δ 6·54 and two singlet signals at 11·56 and 11·22 due to the intramolecularly hydrogen-bonded hydroxy groups at positions 6′ and 2 respectively. Treatment of the depside ester (17) with anhydrous potassium carbonate in dimethyl sulfoxide solution effected the Smiles rearrangement and afforded the corresponding diaryl ether (19) in reasonable yield (60%) (Scheme 3). The structure of (19) followed from the microanalytical and spectroscopic data. The ^{1}H n.m.r. spectrum of (19) exhibited a singlet at δ 5·93 (due to the aromatic proton H 3) and a hydroxy signal at 11·52. The relatively low chemical shift for the aromatic proton indicates that this proton has an 'H-inside' conformation in the diaryl ether product (19).[8,9]

[8] Chester, D. O., Elix, J. A., and Jones, A. J., *Aust. J. Chem.*, 1979, **32**, 1857.
[9] Elix, J. A., Engkaninan, U., Jones, A. J., Raston, C. L., Sargent, M. V., and White, A. H., *Aust. J. Chem.*, 1978, **31**, 2057.

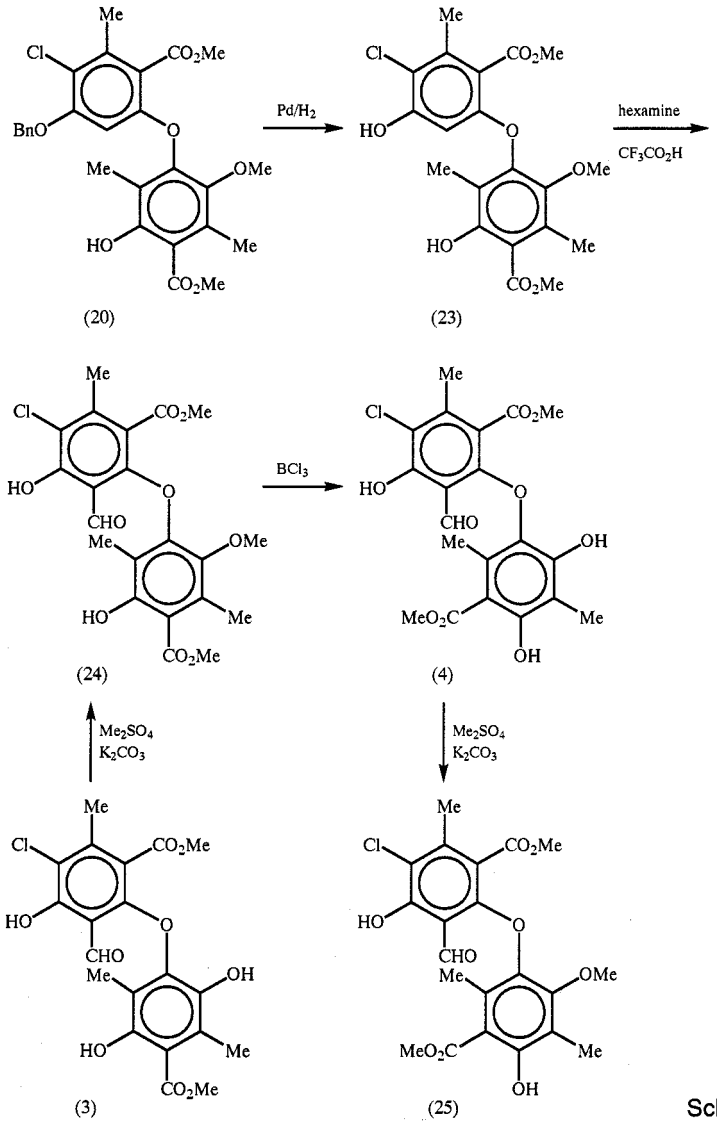
Esterification of (19) was effected with diazomethane to give methyl 4-benzyloxy-5-chloro-2-(5′-hydroxy-2′-methoxy-4′-methoxycarbonyl-3′,6′-dimethylphenoxy)-6-methylbenzoate (20) in good yield (90%). Selective demethylation and debenzylation of (20) was achieved by treatment with boron trichloride to afford a diaryl ether, later shown to be (21). Subsequent formylation of (21) with hexamine and trifluoroacetic acid afforded the ester (4), identical (^{1}H n.m.r., m.s., t.l.c.) with the material prepared by methanolysis of physciosporin (2). Obviously rearrangement had accompanied the penultimate deprotection reaction (with BCl_3) or the final formylation step. In an attempt to establish which step in this synthetic route was accompanied by rearrangement, the diaryl ether (4) was oxidized and decarboxylated. Oxidation of (4) by treatment with sodium chlorite in the presence of sulfamic acid as chlorine scavenger, gave the benzoic acid



Scheme 3

(22). Subsequent decarboxylation of (22) with copper(I) oxide and 2,2′-bipyridine (bpy) in anhydrous diglyme afforded a compound identical with the synthetic intermediate (21). This implied that the rearrangement of the B-ring had taken place upon treatment of compound (20) with boron trichloride or in the subsequent workup procedure.

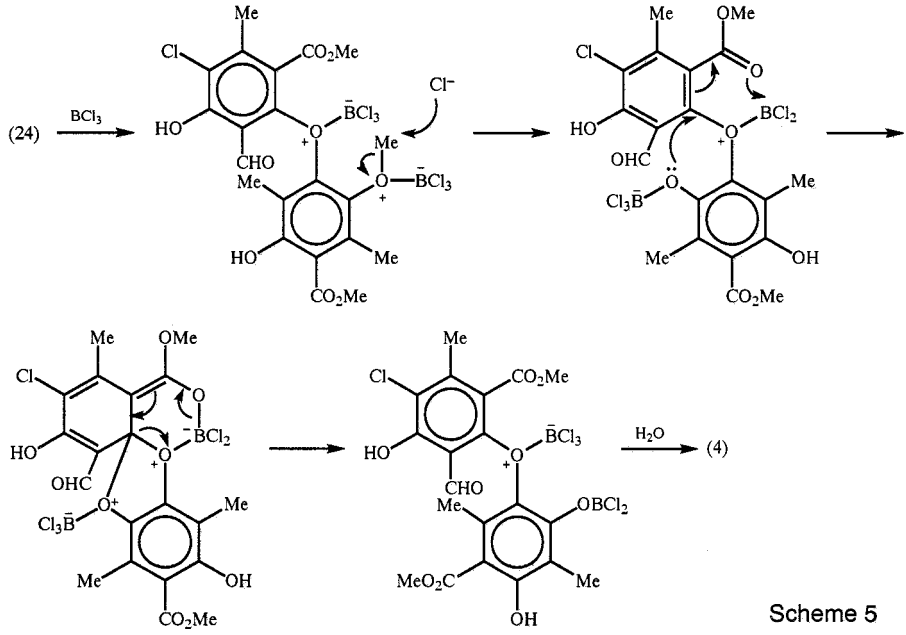
As a result of this observation, an alternative route from compound (20) was undertaken in which debenzylation was effected by catalytic hydrogenolysis prior to the final formylation step (Scheme 4). This procedure afforded the diaryl ether (23), and subsequent formylation by the modified Duff method gave the aldehyde (24) in high yield. Treatment with boron trichloride afforded the diaryl ether (4), thus establishing beyond doubt that rearrangement takes place during this deprotection reaction.



Scheme 4

Treatment of the degradation products (3) and (4) with dimethyl sulfate and anhydrous potassium carbonate gave the corresponding 2′-*O*-methyl derivatives (24) and (25) respectively. The permethyl derivative obtained by methylation of (3) was identical in all respects with the compound (24) prepared by unambiguous synthesis. This in turn established the structure of cyclographin as (1).

The novel rearrangement which accompanied the *O*-demethylation of (20) → (21) and (24) → (4) is of mechanistic interest since it is essentially a Smiles rearrangement effected under acidic conditions. A possible mechanistic explanation is outlined in Scheme 5. Thus, coordination of BCl_3 to the 2′-oxygen atom and cleavage of the methyl group may give rise to a potential nucleophile ($[ArOBCl_3]^-$) which can then attack ring A, an attack facilitated by the neighbouring group participation of the ring A ester group. Subsequent ring opening as indicated (Scheme 5) would lead to the observed rearrangement product.



Scheme 5

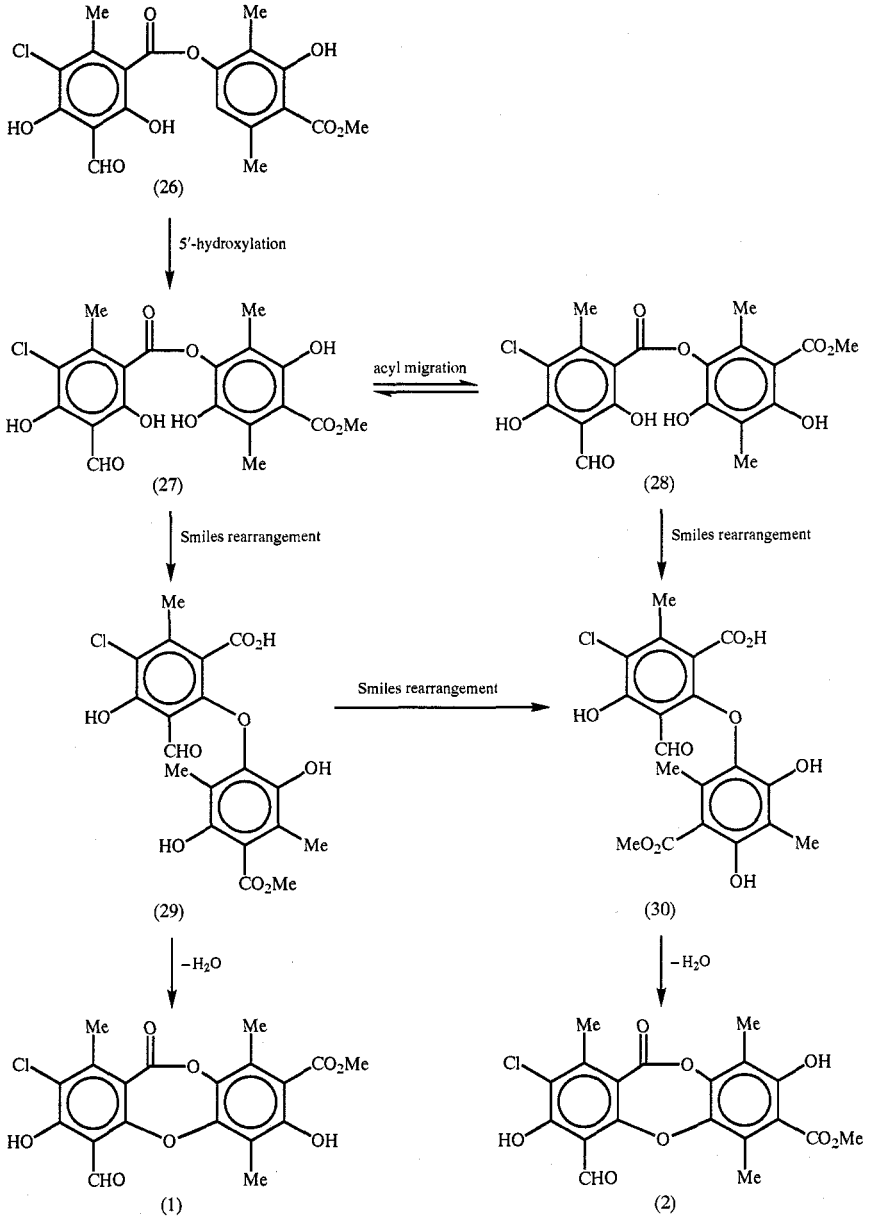
Biosynthetic Implications of the Structure of Cyclographin (1)

The biosynthetic interrelationships between the common lichen polyketides, the depsides, depsidones, diaryl ethers and the dibenzofurans have been a subject of speculation for some time. Following the key discovery by Elix *et al.*[10] that *para*-depsides can readily be converted into the isomeric diaryl ethers through an intramolecular Smiles rearrangement under mild conditions, a plausible route to potential catabolites of such depsides became evident. We have subsequently shown that appropriately hydroxylated *para*-depsides can undergo acyl migration to the corresponding *meta*-depsides,[11] that the latter can be converted into the

[10] Elix, J. A., Jones, A. J., Lajide, L., Coppins, B. J., and James, P. W. *Aust. J. Chem.*, 1987, **37**, 2349.
[11] Elix, J. A., and Gaul, K. L., *Aust. J. Chem.*, 1986, **39**, 613.

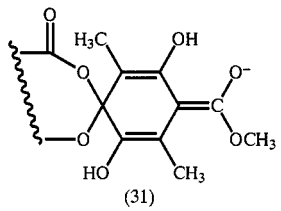
isomeric diaryl ethers by an intramolecular Smiles rearrangement, and that such substituted diaryl ethers can be cyclized to depsidones.[6]

We believe that such a route could be involved in the biosynthesis of cyclographin (1) and physciosporin (2), and have outlined a possible rationale for the formation of these compounds (Scheme 6). Thus 5′-hydroxylation of the common *β*-orcinol *para*-depside chloroatranorin (26), would lead to 5′-hydroxychloroatranorin (27) [= (28)]. Rapid equilibration of this compound through acyl migration would



Scheme 6

lead to a proponderance of the *meta*-depside (28). Several similarly substituted depsides have been recently isolated from lichens.[12,13] However, the isomeric *para*-depside (27) would undergo a much more facile Smiles rearrangement to the corresponding (but thermodynamically less stable) diaryl ether (29). The Smiles rearrangement would be more favourable in this case because of the stabilization of the intermediate anion (31) by the *para*-oriented methoxycarbonyl group. The product diaryl ether (29) could undergo either cyclodehydration to give cyclographin (1) or a second Smiles rearrangement to give a thermodynamically more stable diaryl ether (30) (with *para*-oriented methoxycarbonyl and hydroxy/phenolate anion groups). Subsequent cyclodehydration of (30) would then lead to the common depsidone physciosporin (2).



(31)

Experimental

The general experimental conditions have been reported previously.[14]

Extraction of Catarraphia dictyoplaca

The lichen *Catarraphia dictyoplaca* was collected on bark growing in tropical rain forest in Fiji, Viti Levu, Rewa district, Mount Nakorombamba about 8 km north-west of Suva, altitude 150–400 m, 18° 05′ S, 178° 23′ E, G. Thor 6235 (CANB).

The lichen material (0·30 g, with a small amount of wood) was extracted with acetone (20 ml) in a Soxhlet extractor for 6 h. The solvent was evaporated under reduced pressure and the residue (20 mg) dissolved in acetone and applied to a preparative t.l.c. plate (SiO_2, 20 by 20 by 0·1 cm). The plate was eluted with 40% ethyl acetate/light petroleum and the major band afforded *cyclographin* (1) (6 mg, 2%), which crystallized from the eluent as colourless crystals, m.p. 245–246° (Found: C, 56·2; H, 3·7%; mol. wt, 406·0444. $C_{19}H_{15}ClO_8$ requires C, 56·1; H, 3·7%; mol. wt, 406·0455). ^{1}H n.m.r. ($CDCl_3$) δ 2·27, 2·45, 2·59, 3s, ArMe; 3·98, s, OMe; 10·91, s, CHO; 11·26, 12·92, 2s, OH. Mass spectrum *m/z* 406 (M, 17%), 374 (42), 346 (59), 318 (24), 290 (16), 276 (10), 215 (5), 213 (12), 211 (4), 140 (13), 106 (14), 91 (27), 77 (69), 67 (100).

Methyl 5-Chloro-2-(2′,5′-dihydroxy-4′-methoxycarbonyl-3′,6′-dimethylphenoxy)-3-formyl-4-hydroxy-6-methylbenzoate (3)

Cyclographin (1) (2 mg) was added to anhydrous methanol (5 ml), containing concentrated sulfuric acid (1 drop), and the mixture was stirred at room temperature under an atmosphere of nitrogen for 1 h. The mixture was then poured into water and extracted with ethyl acetate. The ethyl acetate extract was washed with water and dried ($MgSO_4$). After removal of the solvent under reduced pressure, the crude residue was applied to a silica gel plate and eluted with 30% ethyl acetate/light petroleum. The faster moving

[12] Elix, J. A., Barclay, C. E., David, F., Griffin, F. K., Hill, A. H., McConnell, D. B., and Wardlaw, J. H., *Aust. J. Chem.*, 1993, **46**, 301.
[13] Huneck, S., Porzel, A., Schmidt, J., Feige, G. B., and Posner, B., *Phytochemistry*, 1993, **32**, 475.
[14] Elix, J. A., Naidu, R., and Laundon, J. R., *Aust. J. Chem.*, 1992, **45**, 785.

band yielded *methyl 5-chloro-2-(2',5'-dihydroxy-4'-methoxycarbonyl-3',6'-dimethylphenoxy)-3-formyl-4-hydroxy-6-methylbenzoate* (3) as a colourless gum (1 mg, 62%) (Found: C, 54·9; H, 4·7%; mol. wt, 438·0718. $C_{20}H_{19}ClO_9$ requires C, 54·7; H, 4·4%; mol. wt, 438·0718). ^{1}H n.m.r. ($CDCl_3$) δ 2·18, s, 6'-Me; 2·34, s, 3'-Me; 2·43, s, 6-Me; 3·50, 3·91, 2s, OMe; 10·42, s, CHO; 11·32, s, 5'-OH; 12·60, s, 4-OH. Mass spectrum *m/z* 438 (M, 42%), 406 (30), 389 (48), 67 (100). The slower moving band yielded unreacted starting material.

Methyl 5-Chloro-2-(2',4'-dihydroxy-5'-methoxycarbonyl-3',6'-dimethylphenoxy)-3-formyl-4-hydroxy-6-methylbenzoate (4)

(i) *Using sodium methoxide.* Physciosporin (2) (227 mg) was added to a solution of sodium methoxide [derived from sodium (248 mg) in anhydrous methanol (150 ml)] and the reaction mixture was stirred under dry nitrogen at 45° for 1 h. The mixture was then poured into cold 0·1 M hydrochloric acid and extracted with ethyl acetate. The combined organic extract was washed with water and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was separated by radial chromatography with light petroleum/ethyl acetate/acetic acid (70:28:2) as eluent. The major band afforded methyl 5-chloro-2-(2',4'-dihydroxy-5'-methoxycarbonyl-3',6'-dimethylphenoxy)-3-formyl-4-hydroxy-6-methylbenzoate (4) (200 mg, 88%) which crystallized from the eluent as off-white needles, m.p. 225–226° (lit.[1] 226–227°) (Found: mol. wt, 438·0719. Calc. for $C_{20}H_{19}{}^{35}ClO_9$: mol. wt, 438·0718). ^{1}H n.m.r. ($CDCl_3$) δ 2·15, s, 3'-Me; 2·27, s, 6'-Me; 2·32, s, 6-Me; 3·46, 3·93, 2s, OMe; 10·35, s, CHO; 11·81, s, 4'-OH; 12·74, s, 4-OH. Mass spectrum *m/z* 438 (M, 6%), 406 (10), 374 (20), 83 (100).

(ii) *Using methanol and sulfuric acid.* Physciosporin (2) (20 mg) was added to anhydrous methanol (25 ml), containing concentrated sulfuric acid (2 drops), and the mixture was stirred under an atmosphere of nitrogen for 1 h. After workup, the ester (4) (10 mg, 51%) was obtained as off-white needles, identical (m.m.p., t.l.c., ^{1}H n.m.r.) with the material obtained above.

4-Benzyloxy-3-chloro-6-hydroxy-2-methylbenzoic Acid (6)

A solution of 4-benzyloxy-2-hydroxy-6-methylbenzoic acid[15] (5) (1·5 g, 5·8 mmol) in anhydrous ether (24 ml) was treated dropwise at 0° with a solution of sulfuryl chloride (0·47 ml, 5·8 mmol) in anhydrous ether (6 ml) over a 30 min period, and the resulting solution was stirred at room temperature for 22 h. The solvent was then removed under reduced pressure and the residue crystallized from ethyl acetate/light petroleum to afford *4-benzyloxy-3-chloro-6-hydroxy-2-methylbenzoic acid* (6) (1·4 g, 96%) as colourless crystals, m.p. 136° (Found: C, 61·7; H, 4·7. $C_{15}H_{13}ClO_4$ requires C, 61·6; H, 4·5%). ^{1}H n.m.r. ($CDCl_3$) δ 2·72, s, Me; 5·18, s, CH_2; 6·49, s, H5; 7·45, s, Ph; 11·52, s, OH. Mass spectrum *m/z* 292 (M, 10%), 91 (100).

Methyl 4,6-Dibenzyloxy-3-formyl-2,5-dimethylbenzoate (9)

A solution of methyl 3-formyl-4,6-dihydroxy-2,5-dimethylbenzoate[7] (8) (2·5 g, 11·2 mmol) and benzyl bromide (3·79 g, 22·3 mmol) in dimethylformamide (68 ml) and anhydrous potassium carbonate (3·07 g, 22·3 mmol) was stirred at room temperature for 24 h. The mixture was then poured into cold 0·1 M hydrochloric acid and extracted with ethyl acetate. The combined organic extract was washed with water and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was adsorbed on to silica gel and separated by column chromatography (15 by 3 cm) with 5–10% ethyl acetate/light petroleum as eluent. The major fraction afforded *methyl 4,6-dibenzyloxy-3-formyl-2,5-dimethylbenzoate* (9) (2·28 g, 91%) which crystallized from ethyl acetate/light petroleum as colourless microcrystals, m.p. 53–55° (Found: C, 74·8; H, 6·4. $C_{25}H_{24}O_5$ requires C, 74·3; H, 5·9%). ^{1}H n.m.r. ($CDCl_3$) δ 2·23, 2·50, 2s, ArMe; 3·84, s, OMe; 4·92, 4·96, 2s, CH_2O; 7·41, m, Ph; 10·42, s, CHO. Mass spectrum *m/z* 404 (M, 16%), 91 (100).

Methyl 2,4-Dibenzyloxy-5-hydroxy-3,6-dimethylbenzoate (10)

A solution of methyl 4,6-dibenzyloxy-3-formyl-2,5-dimethylbenzoate (9) (2·28 g, 5·64 mmol) in anhydrous dichloromethane (80 ml) was added with stirring over 1 h to a solution of

[15] Bryan, A. J., Elix, J. A., and Norfolk, S., *Aust. J. Chem.*, 1976, **29**, 1079.

85–90% *m*-chloroperoxybenzoic acid (12–14 mmol) in anhydrous dichloromethane (50 ml) at room temperature. The solution was stirred for a further 2 h and the *m*-chlorobenzoic acid collected and washed twice with dichloromethane (10 ml). The filtrate was evaporated under reduced pressure and the residue dissolved in ethyl acetate. The ethyl acetate solution was washed with 10% sodium hydrogen carbonate solution until effervescence ceased and with saturated brine and then dried ($MgSO_4$). The residue on removal of the solvent was then dissolved in methanol (50 ml), and aqueous potassium hydroxide (10%, 50 ml) was added at 0° under a nitrogen atmosphere. The reaction mixture was stirred at this temperature for 1 h, poured into cold 0·1 M hydrochloric acid and then extracted with ethyl acetate. The ethyl acetate extract was washed with water and saturated brine and dried ($MgSO_4$). The solvent was then removed and the residue was separated by radial chromatography (SiO_2) with 5–10% ethyl acetate/light petroleum as eluent. The major band afforded *methyl 2,4-dibenzyloxy-5-hydroxy-3,6-dimethylbenzoate* (10) (1·12 g, 48%) as a colourless sticky gum (Found: C, 73·9; H, 6·4. $C_{24}H_{24}O_5$ requires C, 73·5; H, 6·1%). ^{1}H n.m.r. ($CDCl_3$) δ 2·16, 2·25, 2s, ArMe; 3·84, s, OMe; 4·87, s, CH_2; 5·47, s, OH; 7·44, m, Ph. Mass spectrum *m/z* 392 (M, 20%), 91 (100).

Methyl 2,4-Dibenzyloxy-3,6-dimethyl-5-(propan-2′-yloxy)benzoate (11)

2-Bromopropane (0·032 g, 0·024 ml, 0·26 mmol) was added to a solution of methyl 2,4-dibenzyloxy-5-hydroxy-3,6-dimethylbenzoate (10) (0·10 g, 0·26 mmol) in dimethylformamide (2·72 ml) containing anhydrous potassium carbonate (0·036 g, 0·26 mmol), and the solution was stirred at room temperature for 30 h. The reaction mixture was poured into cold 0·1 M hydrochloric acid and then extracted with ethyl acetate. The ethyl acetate extract was washed with water and saturated brine and dried ($MgSO_4$). The solvent was then removed and the residue purified by radial chromatography (SiO_2) with 5–10% ethyl acetate/light petroleum as eluent. The major band afforded *methyl 2,4-dibenzyloxy-3,6-dimethyl-5-(propan-2′-yloxy)benzoate* (11) (67 mg, 63%) as a colourless oil (Found: C, 75·1; H, 7·2. $C_{27}H_{30}O_5$ requires C, 74·7; H, 6·9%). ^{1}H n.m.r. ($CDCl_3$) δ 1·26, d, $(CH_3)_2CH$; 2·10, 2·22, 2s, ArMe; 3·83, s, OMe; 4·52, m, Me_2CH; 4·85, 4·98, 2s, CH_2O; 7·41, m, Ph. Mass spectrum *m/z* 434 (M, 25%), 91 (100).

Methyl 2,4-Dihydroxy-3,6-dimethyl-5-(propan-2′-yloxy)benzoate (13)

A solution of methyl 2,4-dibenzyloxy-3,6-dimethyl-5-(propan-2′-yloxy)benzoate (11) (65 mg, 0·15 mmol) in ethyl acetate (5 ml) containing 10% palladium on charcoal (10 mg) was stirred at room temperature under an atmosphere of hydrogen for 36 h. The catalyst was then filtered off and the solvent evaporated. The residue afforded *methyl 2,4-dihydroxy-3,6-dimethyl-5-(propan-2′-yloxy)benzoate* (13) (60 mg, 90%) which crystallized from ethyl acetate/cyclohexane as colourless crystals, m.p. 66–67° (Found: C, 61·0; H, 7·1. $C_{13}H_{18}O_5$ requires C, 61·4; H, 7·1%). ^{1}H n.m.r. ($CDCl_3$) δ 1·32, d, $(CH_3)_2CH$; 2·13, 2·24, 2s, ArMe; 3·92, s, OMe; 4·02, m, Me_2CH; 6·29, s, 4-OH; 11·77, s, 2-OH. Mass spectrum *m/z* 254 (M, 12%), 180 (100).

Methyl 2,4-Dibenzyloxy-5-methoxy-3,6-dimethylbenzoate (12)

A solution of methyl 2,4-dibenzyloxy-5-hydroxy-3,6-dimethylbenzoate (10) (0·15 g, 0·38 mmol) and dimethyl sulfate (0·4 mmol) in anhydrous acetone (30 ml) and anhydrous potassium carbonate (0·8 mmol) were stirred and heated under reflux for 22 h. The cooled reaction mixture was then poured into cold 0·1 M hydrochloric acid and extracted with diethyl ether. The ethereal extract was washed with water and saturated brine solution and dried ($MgSO_4$). The solvent was then evaporated and the residue was applied to a column of silica gel and eluted with 10–15% ethyl acetate/light petroleum. The major band afforded *methyl 2,4-dibenzyloxy-5-methoxy-3,6-dimethylbenzoate* (12) (0·14 g, 91%) as a colourless sticky gum (Found: C, 73·5; H, 6·1. $C_{25}H_{26}O_5$ requires C, 73·9; H, 6·4%). ^{1}H n.m.r. ($CDCl_3$) δ 2·13, 2·23, 2s, ArMe; 3·83, s, OMe; 4·84, 5·01, 2s, CH_2O; 7·44, m, Ph. Mass spectrum *m/z* 406 (M, 1%), 91 (100).

Methyl 2,4-Dihydroxy-5-methoxy-3,6-dimethylbenzoate (14)

Hydrogenolysis of methyl 2,4-dibenzyloxy-3-methoxy-2,5-dimethylbenzoate (12) (0·135 g, 0·33 mmol) was effected by treatment with hydrogen and 10% palladium on activated carbon by using the method described above for the preparation of the phenol (13). *Methyl 2,4-dihydroxy-5-methoxy-3,6-dimethylbenzoate* (14) (0·128 g, 92%) crystallized from ethyl acetate/light petroleum as colourless microcrystals, m.p. 138–139° (Found: C, 58·0; H, 6·1. $C_{11}H_{14}O_5$ requires C, 58·3; H, 6·2%). ^{1}H n.m.r. ($CDCl_3$) δ 2·14, 2·45, 2s, ArMe; 3·71, s, ArOMe; 3·93, s, CO_2Me; 6·24, s, 4-OH; 11·80, s, 2-OH. Mass spectrum *m/z* 226 (M, 12%), 83 (100).

Methyl 4-(4′-Benzyloxy-3′-chloro-6′-hydroxy-2′-methylbenzoyloxy)-2-hydroxy-3,6-dimethyl-5-(propan-2″-yloxy)benzoate (15)

N,*N*-Dicyclohexylcarbodiimide (0·45 mmol) was added to a solution of 4-benzyloxy-3-chloro-6-hydroxy-2-methylbenzoic acid (6) (60 mg, 0·21 mmol) and methyl 2,4-dihydroxy-3,6-dimethyl-5-(propan-2′-yloxy)benzoate (13) (50 mg, 0·19 mmol) in anhydrous toluene (1 ml) and anhydrous diethyl ether (1 ml) and the solution was stirred at room temperature for 24 h. The precipitate was then filtered off and the filtrate was evaporated under reduced pressure. The residue was separated by radial chromatography (SiO_2) with 5–20% ethyl acetate/light petroleum as eluent or by p.t.l.c. with 15–20% ethyl acetate/cyclohexane as eluent. The first band yielded 4-benzyloxy-3-chloro-6-hydroxy-2-methylbenzoic anhydride (7 mg, 8%) which crystallized from ethyl acetate/light petroleum as fine yellow needles, m.p. 109–110° (Found: C, 63·7; H, 4·1. $C_{30}H_{24}Cl_2O_7$ requires C, 63·5; H, 4·3%). ^{1}H n.m.r. ($CDCl_3$) δ 2·85, s, Me; 5·24, s, CH_2; 6·67, s, H5; 7·47, m, Ph; 11·49, s, ArOH.

The second band yielded *methyl 4-(4′-benzyloxy-3′-chloro-6′-hydroxy-2′-methylbenzoyloxy)-2-hydroxy-3,6-dimethyl-5-(propan-2″-yloxy)benzoate* (15) (48 mg, 43%) which crystallized from ethyl acetate/light petroleum as pale yellow crystals, m.p. 122–123° (Found: C, 63·9; H, 5·5. $C_{28}H_{29}ClO_8$ requires C, 63·6; H, 5·5%). ^{1}H n.m.r. ($CDCl_3$) δ 1·19, d, $(CH_3)_2CH$; 2·13, s, 6-Me; 2·47, s, 3-Me; 2·85, s, 2′-Me; 3·98, s, OMe; 4·08, m, Me_2CH; 5·19, s, CH_2; 6·55, s, H5′; 7·49, m, Ph; 11·35, 11·55, 2s, OH.

The third band afforded *methyl 2-(4′-benzyloxy-3′-chloro-6′-hydroxy-2′-methylbenzoyloxy)-4-hydroxy-3,6-dimethyl-5-(propan-2″-yloxy)benzoate* (16) (15 mg, 13%) as a colourless gum (Found: C, 63·7; H, 5·3. $C_{28}H_{29}ClO_8$ requires C, 63·6; H, 5·5%). ^{1}H n.m.r. ($CDCl_3$) δ 1·35, d, $(CH_3)_2CH$; 2·08, s, 6-Me; 2·32, s, 3-Me; 2·78, s, 2′-Me; 3·70, s, OMe; 4·21, m, Me_2CH; 5·19, s, CH_2; 6·17, s, 4-OH; 6·53, s, H5′; 7·42, m, Ph; 11·29, s, 6-OH.

Methyl 4-(4′-Benzyloxy-3′-chloro-6′-hydroxy-2′-methylbenzoyloxy)-2-hydroxy-5-methoxy-3,6-dimethylbenzoate (17)

The condensation of 4-benzyloxy-3-chloro-6-hydroxy-2-methylbenzoic acid (6) (70 mg, 0·24 mmol) and methyl 2,4-dihydroxy-5-methoxy-3,6-dimethylbenzoate (14) (50 mg, 0·22 mmol) was effected in the presence of *N*,*N*-dicyclohexylcarbodiimide as described above for the preparation of (15). The residue on removal of solvent was separated by preparative t.l.c. with 20% ethyl acetate/cyclohexane as eluent. The faster moving band afforded 4-benzyloxy-3-chloro-6-hydroxy-2-methylbenzoic anhydride (2 mg, 3%). The slower moving band yielded *methyl 4-(4′-benzyloxy-3′-chloro-6′-hydroxy-2′-methylbenzoyloxy)-2-hydroxy-5-methoxy-3,6-dimethylbenzoate* (17) (39 mg, 41%), which crystallized from ethyl acetate/light petroleum as colourless needles, m.p. 121–122° (Found: C, 62·1; H, 4·9. $C_{26}H_{25}ClO_8$ requires C, 62·4; H, 5·0%). ^{1}H n.m.r. ($CDCl_3$) δ 2·11, s, 6-Me; 2·49, s, 3-Me; 2·85, s, 2′-Me; 3·64, s, OMe; 3·98, s, CO_2Me; 5·20, s, CH_2; 6·54, s, H5′; 7·46, m, Ph; 11·22, s, 2-OH; 11·56, s, 6′-OH. Mass spectrum *m/z* 275 (M, 4%), 241 (2), 226 (32), 194 (100), 179 (53), 166 (25), 151 (22), 123 (20), 91 (32), 83 (81), 67 (47), 55 (33).

4-Benzyloxy-5-chloro-2-(5′-hydroxy-2′-methoxy-4′-methoxycarbonyl-3′,6′-dimethylphenoxy)-6-methylbenzoic Acid (19)

Methyl 4-(4′-benzyloxy-3′-chloro-6′-hydroxy-2′-methylbenzoyloxy)-2-hydroxy-5-methoxy-3,6-dimethylbenzoate (17) (100 mg, 0·20 mmol) was dried by azeotropic distillation with

toluene and then dissolved in a solution containing anhydrous potassium carbonate (28 mg, 0·20 mmol) and anhydrous dimethyl sulfoxide (3 ml). The solution was stirred at room temperature for 48 h. The reaction mixture was then acidified with cold, 0·1 M hydrochloric acid and extracted several times with ethyl acetate. The combined organic fractions were washed with several portions of water and dried ($MgSO_4$) and the solvent was removed under reduced pressure to give the crude acid. The crude residue was separated by preparative t.l.c. with 30% ethyl acetate/toluene as eluent. The major band afforded *4-benzyloxy-5-chloro-2-(5'-hydroxy-2'-methoxy-4'-methoxycarbonyl-3',6'-dimethylphenoxy)-6-methylbenzoic acid* (19) (60 mg, 60%) as a colourless gum (Found: C, 62·6; H, 5·3. $C_{26}H_{25}ClO_8$ requires C, 62·4; H, 5·0%). ^{1}H n.m.r. ($CDCl_3$) δ 1·98, s, 3'-Me; 2·41, s, 6'-Me; 2·53, s, 6-Me; 3·34, s, OMe; 4·03, s, CO_2Me; 4·96, s, CH_2; 5·93, s, H3; 7·25, m, Ph; 11·57, s, OH. Mass spectrum *m/z* 500 (M, 6%), 467 (5), 434 (0·7), 409 (0·4), 194 (0·4), 91 (100).

Methyl 4-Benzyloxy-5-chloro-2-(5'-hydroxy-2'-methoxy-4'-methoxycarbonyl-3',6'-dimethylphenoxy)-6-methylbenzoate (20)

A solution of 4-benzyloxy-5-chloro-2-(5'-hydroxy-2'-methoxy-4'-methoxycarbonyl-3',6'-dimethylphenoxy)-6-methylbenzoic acid (19) (50 mg, 0·10 mmol) in methanol (5 ml) was treated at 0° with excess of an ethereal solution of diazomethane and the mixture was stirred at 0° for 3 min. Excess diazomethane was destroyed by adding 2 drops of glacial acetic acid. Concentration of the solvent under reduced pressure afforded the crude residue which was purified by preparative t.l.c. with ethyl acetate/light petroleum (30:70) as eluent. The major band gave *methyl 4-benzyloxy-5-chloro-2-(5'-hydroxy-2'-methoxy-4'-methoxycarbonyl-3',6'-dimethylphenoxy)-6-methylbenzoate* (20) (45 mg, 90%) as a colourless sticky gum (Found: C, 62·7; H, 5·3. $C_{27}H_{27}ClO_8$ requires C, 62·9; H, 5·3%). ^{1}H n.m.r. ($CDCl_3$) δ 1·91, s, 3'-Me; 2·39, s, 6'-Me; 2·45, s, 6-Me; 3·57, s, OMe; 3·92, 4·02, 2s, CO_2Me; 4·91, s, CH_2; 5·86, s, H3; 7·26, m, Ph; 11·53, s, OH. Mass spectrum *m/z* 514 (M, 5%), 91 (100).

Methyl 5-Chloro-2-(2',4'-dihydroxy-5'-methoxycarbonyl-3',6'-dimethylphenoxy)-4-hydroxy-6-methylbenzoate (21)

A solution of methyl 4-benzyloxy-5-chloro-2-(5'-hydroxy-2'-methoxy-4'-methoxycarbonyl-3',6'-dimethylphenoxy)-6-methylbenzoate (20) (20 mg, 0·04 mmol) in anhydrous dichloromethane (5 ml) was cooled to −10° in an ice–salt bath. Boron trichloride in anhydrous dichloromethane (1 M, 10 ml) was then added and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was then acidified with cold, 0·1 M hydrochloric acid and extracted several times with ethyl acetate. The combined organic fractions were washed with several portions of water and dried ($MgSO_4$) and the solvent was removed under reduced pressure. The crude residue was purified by preparative t.l.c. with ethyl acetate/light petroleum (30:70) as eluent. The major band gave the *title compound* (21) (16 mg, 88%) as a colourless sticky gum (Found: mol. wt, 410·0768. $C_{19}H_{19}{}^{35}ClO_8$ requires mol. wt, 410·0768). ^{1}H n.m.r. ($CDCl_3$) δ 2·03, 2·05, 2s, 3'-Me, 6'-Me; 2·36, s, 6-Me; 3·95, 4·02, 2s, CO_2Me; 5·30, s, H3; 11·94, s, OH. Mass spectrum *m/z* 410 (M, 6%), 57 (100).

Formylation of Ester (21)

The above ester (21) (5 mg, 0·012 mmol) and hexamethylenetetramine (1·96 mg, 0·014 mmol) were dissolved in trifluoroacetic acid (0·25 ml) and cooled to 0°. The mixture was then heated under reflux for 12 h at 90–100° in an atmosphere of nitrogen. The solvent was evaporated under reduced pressure and the residue poured into water (8 ml). After being heated at 60–70° for 6 h, the mixture was cooled in an ice bath and extracted with ethyl acetate. The ethyl acetate extract was washed with water several times and dried ($MgSO_4$). Concentration of the solvent under reduced pressure gave the crude residue which was purified by preparative t.l.c. with 20% ethyl acetate/light petroleum as eluent. The major band gave methyl 5-chloro-2-(2',4'-dihydroxy-5'-methoxycarbonyl-3',6'-dimethylphenoxy)-3-formyl-4-hydroxy-6-methylbenzoate (4), identical (t.l.c., ^{1}H n.m.r., m.s.) with the authentic material prepared above.

3-Chloro-6-(2′,4′-dihydroxy-5′-methoxycarbonyl-3′,6′-dimethylphenoxy)-2-hydroxy-5-methoxycarbonyl-4-methylbenzoic Acid (22)

A solution of methyl 5-chloro-2-(2′,4′-dihydroxy-5′-methoxycarbonyl-3′,6′-dimethylphenoxy)-3-formyl-4-hydroxy-6-methylbenzoate (4) (88 mg, 0·20 mmol) in dioxan (16 ml) and water (4 ml) containing sulfamic acid (33 mg) was stirred while a solution of sodium chlorite (30 mg) in water (0·5 ml) was added. Stirring was continued for 1 h and then the mixture was extracted with ether, washed with water and dried ($MgSO_4$). The solvent was evaporated and the residue crystallized from ethyl acetate/light petroleum to give the *title acid* (22) (60 mg, 69%) as colourless crystals, m.p. 176–177° (Found: mol. wt, 454·0666. $C_{20}H_{19}{}^{35}ClO_{10}$ requires mol. wt, 454·0666). ^1H n.m.r. ($CD_3COCD_3/CDCl_3$) δ 2·20, s, 3′-Me; 2·32, s, 6′-Me; 2·37, s, 6-Me; 3·43, 4·03, 2s, CO_2Me; 11·90, s, OH. Mass spectrum *m/z* 454 (M, 2%), 410 (6), 83 (100).

Decarboxylation of the Acid (22)

The carboxylic acid (22) (55 mg, 0·12 mmol) was dried by azeotropic distillation with toluene and then dissolved in freshly distilled, anhydrous diglyme (9 ml). Cuprous oxide (32 mg) and 2,2′-bipyridine (102 mg) were added, and the mixture was stirred at room temperature for 10 min and then boiled under reflux for 3 h in an atmosphere of nitrogen. The cooled mixture was poured into cold, 0·1 M hydrochloric acid and extracted with ethyl acetate. The organic extract was washed with water and brine and dried ($MgSO_4$). The crude residue obtained after removal of the solvent was purified by preparative t.l.c. with 25% ethyl acetate/light petroleum as eluent. The major band afforded methyl 5-chloro-2-(2′,4′-dihydroxy-5′-methoxycarbonyl-3′,6′-dimethylphenoxy)-4-hydroxy-6-methylbenzoate (21) as a colourless sticky gum, identical (t.l.c., ^1H n.m.r., m.s.) with the material prepared above.

Methyl 5-Chloro-4-hydroxy-2-(5′-hydroxy-2′-methoxy-4′-methoxycarbonyl-3′,6′-dimethylphenoxy)-6-methylbenzoate (23)

Hydrogenolysis of methyl 4-benzyloxy-5-chloro-2-(5′-hydroxy-2′-methoxy-4′-methoxycarbonyl-3′,6′-dimethylphenoxy)-6-methylbenzoate (20) (40 mg, 0·078 mmol) was effected by treatment with hydrogen and 10% palladium on carbon by using the method described for the synthesis of the ester (13) above. The *ester* (23) was obtained as a colourless gum (35 mg, 88%) (Found: C, 56·8; H, 4·9. $C_{20}H_{21}ClO_8$ requires C, 56·5; H, 4·9%). ^1H n.m.r. ($CDCl_3$) δ 2·06, s, 3′-Me; 2·38, s, 6′-Me; 2·45, s, 6-Me; 3·63, s, OMe; 3·94, 3·98, 2s, CO_2Me; 5·99, s, 4-OH; 11·53, s, 5′-OH. Mass spectrum *m/z* 424 (M, 24%), 67 (100).

Methyl 5-Chloro-3-formyl-4-hydroxy-2-(5′-hydroxy-2′-methoxy-4′-methoxycarbonyl-3′,6′-dimethylphenoxy)-6-methylbenzoate (24)

(i) A solution of methyl 5-chloro-4-hydroxy-2-(5′-hydroxy-2′-methoxy-4′-methoxycarbonyl-3′,6′-dimethylphenoxy)-6-methylbenzoate (23) (20 mg, 0·047 mmol) and hexamethylenetetramine (7·84 mg, 0·056 mmol) were dissolved in trifluoroacetic acid (1 ml) at 0°. The mixture was then heated under reflux for 10 h at 90–100° in an atmosphere of nitrogen. The solvent was evaporated under reduced pressure and the residue poured into water (8 ml). After being heated at 60–70° for 6 h, the mixture was cooled in an ice bath and extracted with ethyl acetate. The ethyl acetate extract was washed with water several times and dried ($MgSO_4$). Concentration of the solvent under reduced pressure gave the crude residue which was separated by preparative t.l.c. with 20% ethyl acetate/light petroleum as eluent. The major band afforded the *title ester* (24) (20 mg, 95%) as a pale yellow sticky gum (Found: C, 56·0; H, 5·1. $C_{21}H_{21}ClO_9$ requires C, 55·7; H, 4·8%). ^1H n.m.r. ($CDCl_3$) δ 2·20, s, 3′-Me; 2·31, s, 6′-Me; 2·40, s, 6-Me; 3·34, 3·37, 3·97, 3s, OMe; 10·42, s, CHO; 11·56, s, 5′-OH; 12·65, s, 4-OH. Mass spectrum *m/z* 452 (M, 35%), 420 (41), 211 (12), 178 (37), 149 (11), 91 (16), 83 (22), 77 (25), 67 (100).

(ii) Methyl 5-chloro-2-(2′,5′-dihydroxy-4′-methoxycarbonyl-3′,6′-dimethylphenoxy)-3-formyl-4-hydroxy-6-methylbenzoate (3) (1 mg) obtained by methanolysis of natural cyclographin (1) was methylated with dimethyl sulfate (2 mg), anhydrous acetone (1 ml) and anhydrous potassium carbonate (10 mg) for 2 h as described above in the synthesis of the ester (12). After

the usual workup, evaporation of the solvent gave an oil which was purified by preparative t.l.c. with 10% ethyl acetate/cyclohexane as eluent. The major band afforded the ester (24) (0·6 mg, 64%) as a pale yellow sticky gum, identical (t.l.c., ^{1}H n.m.r., m.s.) with the synthetic compound above.

Rearrangement of Methyl 5-Chloro-3-formyl-4-hydroxy-2-(5′-hydroxy-2′-methoxy-4′-methoxycarbonyl-3′,6′-dimethylphenoxy)-6-methylbenzoate (24)

A solution of methyl 5-chloro-3-formyl-4-hydroxy-2-(5′-hydroxy-2′-methoxy-4′-methoxycarbonyl-3′,6′-dimethylphenoxy)-6-methylbenzoate (24) (2 mg) in anhydrous dichloromethane (1 ml) was cooled to −10° in an ice–salt bath. Boron trichloride in anhydrous dichloromethane (1 M, 1 ml) was then added and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was then acidified with cold, 0·1 M hydrochloric acid and extracted several times with ethyl acetate. The combined organic fractions were washed with several portions of water and dried ($MgSO_4$), and the solvent was removed under reduced pressure. The crude residue was purified by preparative t.l.c. with ethyl acetate/light petroleum (30 : 70) as eluent. The major band gave the ester (4) (1·6 mg, 80%) as colourless crystals, identical (m.p., t.l.c., ^{1}H n.m.r., m.s.) with the authentic material prepared above.

Methyl 5-Chloro-3-formyl-4-hydroxy-2-(4′-hydroxy-2′-methoxy-5′-methoxycarbonyl-3′,6′-dimethylphenoxy)-6-methylbenzoate (25)

Methyl 5-chloro-2-(2′,4′-dihydroxy-5′-methoxycarbonyl-3′,6′-dimethylphenoxy)-3-formyl-4-hydroxy-6-methylbenzoate (4) (25 mg) obtained by methanolysis of natural physciosporin (2) was methylated with dimethyl sulfate, acetone and anhydrous potassium carbonate for 2 h as described above. After the usual workup, evaporation of the solvent gave an oil which was purified by preparative t.l.c. with 10% ethyl acetate/cyclohexane as eluent. The major band afforded *methyl 5-chloro-3-formyl-4-hydroxy-2-(4′-hydroxy-2′-methoxy-5′-methoxycarbonyl-3′,6′-dimethylphenoxy)-6-methylbenzoate* (25) (10 mg, 44%) as a pale yellow sticky gum (Found: C, 56·2; H, 5·1. $C_{21}H_{21}ClO_9$ requires C, 55·7; H, 4·8%). ^{1}H n.m.r. ($CDCl_3$) δ 2·15, s, 6′-Me; 2·25, s, 3′-Me; 2·45, s, 6-Me; 3·27, 3·57, 3·99, 3s, OMe; 10·49, s, CHO; 11·61, s, 4′-OH; 12·72, s, 4-OH. Mass spectrum *m/z* 452 (M, 18%), 57 (100).

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