Studies in the Biochemistry of Micro-organisms

85. CYCLOPOLIC AND CYCLOPALDIC ACIDS, METABOLIC PRODUCTS OF *PENICILLIUM CYCLOPIUM* WESTLING

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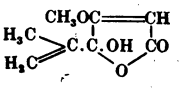
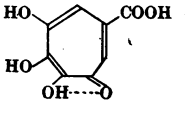
The genus *Penicillium* has been separated by Raper & Thom (1949) in their *Manual of the Penicillia* into four major sections, Monoverticillata, Asymmetrica, Biverticillata-Symmetrica and Polyverticillata. The section Asymmetrica is further divided into five subsections: Divaricata, Velutina, Lanata, Funiculosa and Fasciculata. The subsection Asymmetrica-Fasciculata, in its turn, is subdivided into ten series, one of which, the *P. cyclopium* series, contains four accepted species and one variety, i.e. *P. cyclopium* Westling, *P. cyclopium* West. var. *echinulatum* Raper & Thom, *P. puberulum* Bainier, *P. martensii* Biourge, and *P. aurantio-virens* Biourge.

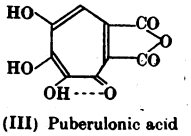
The *P. cyclopium* series has proved to be of considerable biochemical interest. In 1913, Alsberg & Black isolated from laboratory cultures of *P. puberulum* a metabolic product which they named penicillic acid. Birkinshaw, Oxford & Raistrick (1936) isolated from Westling's type strain of *P. cyclopium* considerably larger yields of penicillic acid and established its molecular structure (structure I). Birkinshaw & Raistrick (1932), working with Alsberg & Black's strain of *P. puberulum*, showed that, after almost 20 years in laboratory culture, this strain now gave much smaller yields of penicillic acid than were reported by Alsberg & Black. In addition, however, two new metabolic products, puberulic and puberulonic acids, were isolated. The same two acids were also isolated by Birkinshaw & Raistrick (1932) from *P. aurantio-virens* and by Oxford, Raistrick & Smith (1942*b*) from *P. johannioli* Zaleski (= *P. martensii* Biourge). Penicillic acid, puberulic acid and puberulonic acid have significant antibacterial activity, particularly against Gram-positive bacteria (Oxford, Raistrick & Smith, 1942*a*, *b*). Birkinshaw & Raistrick (1932) and Barger & Dorrer (1934) prepared a number of derivatives and breakdown products of puberulic and puberulonic acids but were unable to advance plausible structural formulae for them. Dewar (1945), in a novel attempt to interpret this experimental evidence and additional evidence on stipitatic acid (Birkinshaw, Chambers & Raistrick, 1942), suggested that puberulic acid might contain a seven-carbon ring and be in fact a derivative of what he proposed to call tropolone (*cyclo*heptatrienolone or hydroxy-*cyclo*heptatrienone), at that time unknown, puberulic acid thus becoming a dihydroxytropolonecarboxylic acid of undetermined orientation. Corbett, Johnson & Todd (1950*a*) have confirmed this view and have shown that puberulic acid has structure (II). The structure of puberulonic acid, which on heating with dilute sulphuric acid loses a molecule of carbon dioxide and is converted into puberulic acid (Corbett, Hassall, Johnson & Todd, 1950), seems now to be satisfactorily settled. Three possible structural formulae were advanced by Corbett *et al.* (1950*a*), but doubt was thrown on these formulae by Aulin-Erdtman (1950) who prefers structure (III) (see also Cook & Loudon, 1951). This structure has been confirmed by Aulin-Erdtman & Theorell (1950) and has now been accepted by Johnson, Sheppard & Todd (1951).



(I) Penicillic acid



(II) Puberulic acid



(III) Puberulonic acid

Other naturally occurring substances which have been shown to contain the tropolone nucleus are stipitatic acid from *P. stipitatum* Thom (Birkinshaw, *et al.* 1942; Corbett, Johnson & Todd, 1950*b*), the α-, β- and γ-thujaplicins from *Thuja plicata*, the western red cedar tree (Erdtman & Gripenberg, 1948*a*, *b*), colchicine (Dewar, 1945; Lettré, 1947; Arnstein, Tarbell, Huang & Scott, 1948) and purpurogallin which occurs as the glycoside eriophyesin (Barltrop & Nicholson, 1948; Haworth, Moore & Pauson, 1948).

We have examined a number of strains of *P. cyclopium*, a species which is well defined morphologically, but many of the strains of this ubiquitous species have shown no biochemical features of interest. We have, however, obtained from each of two strains, recently isolated from natural sources, two hitherto undescribed mould metabolic products for which we propose the names cyclopolic acid and cyclopaldic acid, and it is the purpose of the present communication to describe these substances. The two strains of *P. cyclopium* used, while indubitably strains of *P. cyclopium* Westling morphologically, are quite different in appearance. Strain no. 77 is a typical green sporing strain. Strain no. 85, on the other hand, while morphologically similar to no. 77 in all other respects, differs from it in its total lack of green conidial pigment. It is almost pure white in colour and has been described by our colleague, Mr G. Smith (see Smith, 1951), as a new variety, *P. cyclopium* Westling var. *album* G. Smith.

CYCLOPOLIC ACID

Cyclopolic acid, $C_{11}H_{12}O_6$, is closely related, structurally, to cyclopaldic acid, $C_{11}H_{10}O_6$, as is indicated *inter alia* by the fact that when cyclopolic acid is oxidized with potassium periodate in hot dilute sulphuric acid solution cyclopaldic acid is formed in fairly good yield. For this reason, and also because cyclopolic acid is formed by both strains nos. 85 and 77 in much greater yields than is cyclopaldic acid, most of the experimental work on the chemistry and structure of these two acids was carried out on cyclopolic acid.

Cyclopolic acid, $C_{11}H_{12}O_6$, forms colourless plates, m.p. 147–148° (decomp.). It has no optical activity. It contains one methoxyl group and one methyl group attached to carbon. It dissolves at once, with liberation of carbon dioxide, in aqueous sodium bicarbonate and forms a colourless solution both in this alkali and in aqueous sodium hydroxide. It titrates conductometrically as a dibasic acid. An aqueous solution of cyclopolic acid gives an intense stable purple colour, almost indistinguishable from that given by salicylic acid, with aqueous ferric chloride; immediately decolorizes bromine water with the formation of a precipitate soluble in excess of the reagent; quickly gives a deep-yellow crystalline precipitate with Brady's reagent (0·32% 2:4-dinitrophenylhydrazine in 2N-hydrochloric acid); gives a negative reaction with Schiff's reagent; reduces ammoniacal silver nitrate solution on warming.

The following functional derivatives have been prepared: cyclopolic acid mono-2:4-dinitrophenylhydrazone, $C_{11}H_{12}O_5{:}N.NH.C_6H_3(NO_2)_2$, deep-yellow microprisms, m.p. 190·5–191° (decomp.); cyclopolic acid thiosemicarbazone, $C_{11}H_{12}O_5{:}N.NH.CS.NH_2$, colourless microprisms, m.p. 180·5–182° (decomp.), which are readily soluble in cold aqueous sodium bicarbonate and give in ethanolic solution an orange colour with ethanolic ferric chloride; methyl cyclopolate monomethyl ether, $C_{10}H_{10}O_3(OCH_3).(COOCH_3)$, m.p. 87·5–88°, by treatment of cyclopolic acid with ethereal diazomethane. It gives no colour with aqueous ferric chloride, but quickly gives a yellow precipitate with Brady's reagent. Cyclopolic acid monomethyl ether mono-2:4-dinitrophenylhydrazone, $C_{12}H_{14}O_5{:}N.NH.C_6H_3(NO_2)_2$, yellow needles, m.p. 287° (decomp.), is soluble in cold saturated aqueous sodium bicarbonate.

When cyclopolic acid is heated above its melting point, or when a solution of cyclopolic acid in 2N-sulphuric acid is boiled, the acid is converted, with the loss of one molecule of water, into a colourless crystalline compound, cyclopolide, $C_{11}H_{10}O_5$, m.p. 169°. On acetylation, either with acetic anhydride and anhydrous sodium acetate or with acetic anhydride and pyridine, cyclopolic acid gives, in good yield, a triacetate, $C_{11}H_9O_3(O.CO.CH_3)_3$, m.p. 117°, which, unlike the parent substance, does not dissolve in cold 2N-sodium hydroxide, gives no colour with ethanolic ferric chloride nor any precipitate with Brady's reagent. This triacetate, called for convenience cyclopolide triacetate, on hydrolysis with boiling 2N-sulphuric acid, gives three molecules of acetic acid and an almost theoretical yield of cyclopolide. Cyclopolide is readily soluble in cold aqueous sodium bicarbonate, gives a pale-yellow solution in cold 2N-sodium hydroxide, a deep ruby-red colour very similar to that given by cyclopaldic acid, with ethanolic ferric chloride and an immediate orange precipitate with Brady's reagent. The following derivatives of cyclopolide have been prepared: cyclopolide monomethyl ether, $C_{11}H_9O_4(OCH_3)$, m.p. 169–170°, insoluble in cold aqueous 2N-sodium hydroxide and giving no colour with ethanolic ferric chloride; mono-2:4-dinitrophenylhydrazone of cyclopolide monomethyl ether, $C_{12}H_{12}O_4{:}N.NH.C_6H_3(NO_2)_2$, deep-orange needles which have no sharp melting point; diacetate of cyclopolide monomethyl ether, $C_{12}H_{12}O_4(O.CO.CH_3)_2$, m.p. 133·5–134°, which, unlike cyclopolide monomethyl ether, no longer gives a precipitate with Brady's reagent.

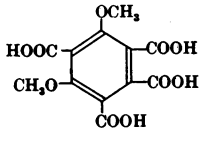
The experimental evidence presented so far justifies the working hypothesis that there are present in the molecule of cyclopolic acid the following groups: one OCH_3 group actually estimated; one C-CH_3 group actually estimated in both cyclopolic acid and cyclopolide; one OH group to account for the intense purple ferric chloride colour given by cyclopolic acid and the absence of this colour in methyl cyclopolate monomethyl ether; one carbonyl group, probably a CHO group, explaining the formation of cyclopolic acid thiosemicarbazone

and mono-2:4-dinitrophenylhydrazone; one COOH group indicated by conductometric titration, which accounts for the liberation by cyclopolic acid of carbon dioxide from aqueous sodium bicarbonate and which is esterified in methyl cyclopolate monomethyl ether; one CH_2OH group, vicinal to the COOH group, to account for the formation of cyclopolide with the loss of one molecule of water. On this hypothesis the formula for cyclopolic acid may be written $C_6.(OCH_3).(CH_3).(OH).(CHO).(COOH).(CH_2OH) = C_{11}H_{12}O_6$. Cyclopolic acid would then be a hexasubstituted benzene derivative, four of the substituents having C—C linkages and two of them having C—O linkages. Further, cyclopolide, which arises from cyclopolic acid by the loss of water, would involve the formation of the phthalide grouping,

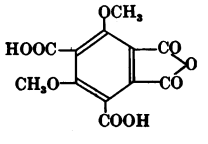
and the formation of a triacetate from cyclopolic acid would involve the closing of the phthalide ring, the acetylation of the OH group and the conversion of the CHO group into $CH(O.CO.CH_3)_2$, a similar mechanism explaining the formation of a diacetate from cyclopolide monomethyl ether.

The correctness of this hypothesis has been proved by a number of degradation experiments.

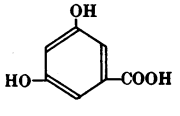
(*a*) Methyl cyclopolate monomethyl ether was oxidized with hot alkaline potassium permanganate. The main oxidation product, obtained in fairly good yield, was a tetracarboxylic acid, $C_{12}H_{10}O_{10}$ (IV), which was analysed as its anhydride and tetramethyl ester. The anhydride, $C_{12}H_8O_9$ (V),



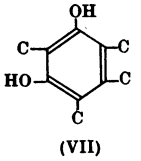
(IV)



(V)



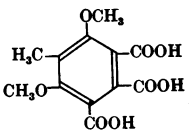
(VI)



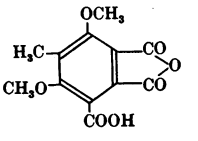
(VII)

colourless microprisms, m.p. 225·5–228° (decomp.), contained two methoxyl groups and titrated as a tetrabasic acid (di-acid mono-anhydride). The tetramethyl ester of (IV), $C_{16}H_{18}O_{10}$, a waxy solid, m.p. 44–46°, contained six methoxyl groups. The structure of the oxidation acid, $C_{12}H_{10}O_{10}$ (IV), 4:6-dimethoxybenzene-1:2:3:5-tetracarboxylic acid (cf. Birkinshaw, Raistrick & Ross, 1952), was conclusively proved as follows. It was heated in a stream of carbon dioxide-free nitrogen with hydriodic acid (*d*, 1·7) and red phosphorus. The acid was thus demethylated, three molecules of carbon dioxide were evolved and α-resorcylic acid (3:5-dihydroxybenzoic acid) (VI) was isolated in good yield and identified by comparison of it and of its dimethyl ether with authentic synthetic specimens. It therefore follows that cyclopolic acid must contain the skeleton structure (VII). The structure of the anhydride (V) follows because of the symmetrical nature of the free acid (IV).

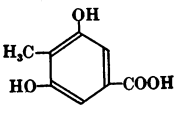
(*b*) Methyl cyclopolate monomethyl ether was oxidized with cold alkaline potassium permanganate. The main oxidation product was a tricarboxylic acid, $C_{12}H_{12}O_8$ (VIII), which was analysed as its anhydride, $C_{12}H_{10}O_7$ (IX). The anhydride, m.p. 198·5–199·5°, contained two methoxyl groups and titrated as a tribasic acid (mono-acid mono-anhydride). The structure of the oxidation acid,



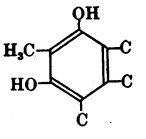
(VIII)



(IX)



(X)



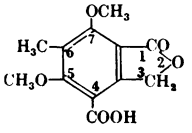
(XI)

$C_{12}H_{12}O_8$ (VIII), 4:6-dimethoxy-5-methylbenzene-1:2:3-tricarboxylic acid, was conclusively proved as follows. On heating with hydriodic acid and red phosphorus the acid was demethylated, two molecules of carbon dioxide were evolved and 3:5-dihydroxy-*p*-toluic acid (X) was isolated and identified by comparison of it and of its methyl ester with authentic synthetic specimens. The skeleton structure of cyclopolic acid may therefore be expanded to (XI). The structure of the anhydride (IX) follows because of the symmetrical nature of the free acid (VIII).

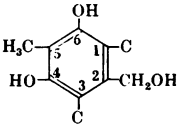
(*c*) 3:5-Dihydroxy-*p*-toluic acid (X) was also isolated from the products of the fusion of cyclopolic acid with potassium hydroxide at 300°. It was identified by comparison of it, of its methyl ester and of the diacetate of its methyl ester with authentic synthetic specimens.

(*d*) Cyclopolide monomethyl ether was oxidized with cold alkaline potassium permanganate. 4-Carboxy-5:7-dimethoxy-6-methylphthalide (XII) was obtained in good yield as fine colourless needles, m.p. 198–199°, not depressed on admixture with an authentic synthetic specimen, m.p. 198–198·5° (for

synthesis see p. 627). This oxidation product is also identical with the methyl ether of *iso*cyclopaldic acid (see p. 621). The skeleton structure of cyclopolic acid may therefore be further expanded to (XIII).

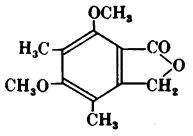


(XII)

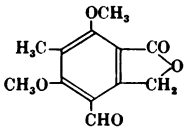


(XIII)

(*e*) Cyclopolic acid was submitted to a Clemmensen reduction by boiling in aqueous hydrochloric acid solution with mercury-amalgamated zinc. The resulting reduction product, $C_{11}H_{12}O_4$, which no longer gave a precipitate with Brady's reagent or any colour with ferric chloride, was obtained as colourless lustrous needles, m.p. 202·5–203·5°. This substance, on methylation with dimethyl sulphate, acetone and anhydrous potassium carbonate, gave 5:7-dimethoxy-4:6-dimethylphthalide, $C_{12}H_{14}O_4$, (XIV), as colourless needles, m.p. 101·5–102°, not depressing the melting point of an authentic synthetic specimen (for synthesis see p. 627). It is clear that in this reduction the CHO group in cyclopolic acid is reduced to CH_3. Hence, since it has already been shown that the methyl group originally present in cyclopolic acid is in position 5 in (XIII), the CHO group in cyclopolic acid which gives rise to the second CH_3 group in (XIV) must be in position 3 in (XIII). Cyclopolide monomethyl ether must, therefore, have structure (XV), and the methyl ether of cyclopolic acid must have structure (XVI).



(XIV)



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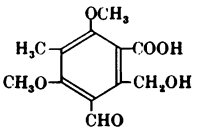
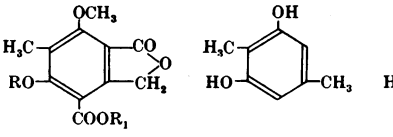


(XVI)

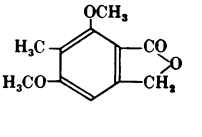
(*f*) The general orientation of cyclopolic acid was confirmed and its relation to cyclopaldic acid was indicated by a different line of approach. Cyclopolic acid was heated in air with potassium hydroxide and a little water at 150° until the melt ceased to give a precipitate with Brady's reagent. The resulting oxidation product, *iso*cyclopaldic acid, $C_{11}H_{10}O_6$ (XVII; $R=R_1=H$), was isolated as colourless needles, m.p. 223·5–224° (decomp.). The same acid was also obtained by heating cyclopaldic acid for a short time with aqueous 2N-sodium hydroxide. *iso*Cyclopaldic acid forms a monomethyl ether, colourless needles, m.p. 198–199°, which did not depress the melting point of authentic synthetic 4 - carboxy - 5 : 7 - dimethoxy - 6 - methylphthalide (XVII; $R=CH_3$, $R_1=H$), m.p. 198–198·5° (for synthesis see p. 627). This is the same monomethyl ether as is formed by the oxidation of cyclopolide monomethyl ether with cold alkaline potassium permanganate (see § *d*, p. 612, structure XII). *iso*Cyclopaldic acid also forms a monomethyl ether methyl ester, colourless needles, m.p. 139–139·5°, which did not depress the melting point of authentic synthetic 4 - carbomethoxy - 5 : 7 - dimethoxy - 6 - methylphthalide (XVII; $R=R_1=CH_3$), m.p. 139–139·5° (for synthesis see p. 627).

When *iso*cyclopaldic acid (XVII; $R=R_1=H$) was heated in nitrogen with hydriodic acid and red phosphorus it was demethylated, two molecules of carbon dioxide were evolved and the degradation product was identified as β-orcinol (XVIII) by comparison of it and of its diacetate with authentic synthetic specimens. On the other hand, when *iso*cyclopaldic acid was decarboxylated by heating with copper chromite and quinoline only one molecule of carbon dioxide was evolved and the methylated decarboxylated product was identified as 5:7-dimethoxy-6-methylphthalide (XIX), colourless needles, m.p. 171·5–172·5°, not depressed on admixture with an authentic synthetic specimen, m.p. 172–172·5° (for synthesis see p. 627). 5:7-Dimethoxy-6-methylphthalide (XIX) is of particular interest since it links cyclopolic acid and cyclopaldic acid with the lichen acid, barbatolic acid (XX). Suominen (1939), who isolated barbatolic acid from the lichen *Alectoria implexa* (Hoffm.) Nyl. f. *fuscidula* Arn., assigned structure (XX) to it. He esterified the carboxyl group in ring A with diazomethane and reduced the resulting methyl ester with zinc dust and hydriodic acid at 70°

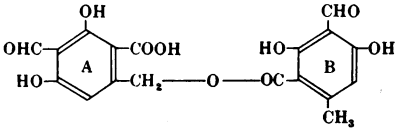


(XVII)

(XVIII)



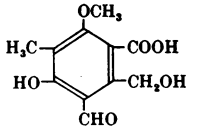
(XIX)



(XX)
Barbatolic acid

obtaining a reduction product, $C_{19}H_{20}O_8$, m.p. 189°, in which the aldehyde groups in rings A and B were reduced to methyl groups. The four hydroxyl groups in rings A and B were then methylated with diazomethane and the resulting methylated product was hydrolysed with ethanolic potassium hydroxide. He identified one of the resulting hydrolysis products as 5:7-dimethoxy-6-methylphthalide. Through the courtesy of Dr E. E. Suominen we received a specimen of the reduction product $C_{19}H_{20}O_8$, m.p. 189°, and have repeated his degradation process with some minor modifications. We isolated 5:7-dimethoxy-6-methylphthalide, m.p. 171·5°, not depressing the melting point of our synthetic 5:7-dimethoxy-6-methylphthalide, m.p. 172–172·5°, which has not previously been synthesized. Suominen (1939) gives the m.p. as 173·5°.

Having established the structural formula for the monomethyl ether of cyclopolic acid as (XVI) it now remains to decide which of the methoxyl groups—whether that in position 4 or that in position 6 in (XVI)—in this monomethyl ether is present as a



(XXI)
Cyclopolic acid

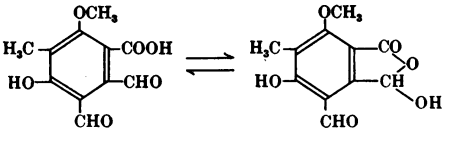
free hydroxyl group in cyclopolic acid itself. We believe that cyclopolic acid has structure (XXI), but defer consideration of the experimental evidence for this belief until the structural formula for cyclopaldic acid has been discussed.

CYCLOPALDIC ACID

Cyclopaldic acid, $C_{11}H_{10}O_6$, forms colourless fluffy needles, m.p. 224–225°, which sublime readily in a high vacuum at 170°. It contains one methoxyl group and one methyl group attached to carbon. It dissolves slowly in cold aqueous sodium bicarbonate and forms a yellow solution both in this alkali and in aqueous sodium hydroxide, in which it is quickly soluble. It titrates conductometrically as a dibasic acid. An aqueous solution of cyclopaldic acid gives with ferric chloride an intense ruby-red colour with no suggestion of blue; decolorizes bromine water without the formation of a precipitate; quickly gives a gelatinous orange precipitate with Brady's reagent; gives a negative reaction with Schiff's reagent. When solid cyclopaldic acid is exposed to ammonia vapour it rapidly turns brown, and if then dissolved in aqueous ammonia (*d*, 0·880) it gives an intense purple colour slowly fading to orange with a yellow fluorescence. Cyclopolic acid does not give this characteristic reaction.

The close structural relationship of cyclopaldic acid, $C_{11}H_{10}O_6$, to cyclopolic acid, $C_{11}H_{12}O_6$, is evident from the following experimentally established facts: (i) When cyclopolic acid is oxidized with potassium periodate in hot dilute sulphuric acid solution cyclopaldic acid is formed in fairly good yield. (ii) Cyclopaldic acid is smoothly converted into the isomeric *iso*cyclopaldic acid, $C_{11}H_{10}O_6$ (XVII; $R = R_1 = H$) when its solution in aqueous 2N-sodium hydroxide is boiled for a short time. The same compound is formed from cyclopolic acid by oxidation in air at 150° by heating with potassium hydroxide and a little water. (iii) Both methyl oxycyclopaldate methyl ether (XXVII) (see p. 615) and methyl cyclopolate monomethyl ether, on oxidation with hot alkaline potassium permanganate, yield the same oxidation product, 4:6-dimethoxybenzene-1:2:3:5-tetracarboxylic acid, (IV). (iv) Methyl oxycyclopaldate methyl ether, on mild oxidation with potassium permanganate and subsequent hydrolysis, yields 4:6-dimethoxy-5-methylbenzene-1:2:3-tricarboxylic acid (VIII), identical with the mild permanganate oxidation product from monomethyl cyclopolate methyl ether.

The simplest, and probably the only feasible, explanation for the formation of cyclopaldic acid from cyclopolic acid by the loss of two atoms of hydrogen, is that the carbinol group in cyclopolic acid (XXI) is converted into an aldehyde group in cyclopaldic acid. We therefore propose structure (XXII) for cyclopaldic acid, and, since some of its reactions are more readily explained by the tautomeric hydroxyphthalide form (*b*) than by the dialdehyde form (*a*), it becomes necessary to postulate that cyclopaldic acid may function in either form according to experimental conditions.

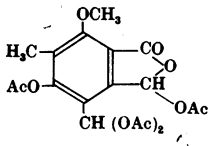


(*a*) (*b*)
(XXII) Cyclopaldic acid

The formation of the following derivatives and degradation products of cyclopaldic acid is readily explained in some cases by tautomeric form (*a*) and in other cases by tautomeric form (*b*).

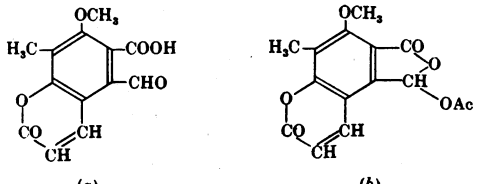
When cyclopaldic acid is heated at 150° for 0·5 hr. with anhydrous sodium acetate and acetic anhydride there is formed an acetylation product, $C_{19}H_{20}O_{11}$, in colourless needles, m.p. 159°. This substance, on acid hydrolysis, gives four molecules of acetic acid and is clearly the tetra-acetyl derivative of $C_{11}H_{10}O_6 + H_2O$. Unlike the parent substance, it no longer gives a ferric colour. Its structure (XXIII)

is readily derived from structure (XXIIb) for cyclopaldic acid.



(XXIII)

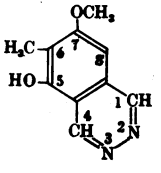
When a similar acetylation mixture was held at 180–190° for 6·5 hr. an entirely different product, $C_{15}H_{12}O_7$ (XXIVb), derived from (XXIIb), was obtained in colourless needles, m.p. 197·5–198·5°. On acid hydrolysis, this substance yielded one molecule of acetic acid and a substituted coumarin, $C_{13}H_{10}O_6$, in colourless crystals, m.p. 275–277°, for which we propose structure (XXIVa), derived from (XXIIa). It dissolves slowly in aqueous sodium bicarbonate to give a colourless solution, and at once in cold aqueous sodium carbonate or sodium hydroxide to give a yellow solution. It gives no ferric colour, but forms a yellow crystalline derivative with Brady's reagent in aqueous ethanolic solution.



(a) (b)

(XXIV)

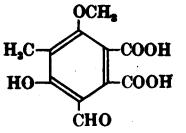
When a mixture of cyclopaldic acid, hydrazine and water is boiled for 15 min. there is formed, with the loss of one molecule of carbon dioxide, a condensation product, $C_{10}H_{10}O_2N_2$ (XXV), in pale-yellow prisms, m.p. 260–262° (decomp.). This



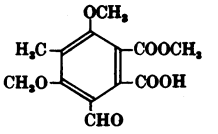
(XXV)

substance, which is a base forming a crystalline hydrochloride, gives a red-brown colour with ferric chloride in ethanol, but no precipitate with Brady's reagent. Its formation from cyclopaldic acid structure (XXIIa) is readily explained, by assigning to it structure (XXV), as a derivative of phthalazine (4:5-benzopyridazine), i.e. 5-hydroxy-7-methoxy-6-methylphthalazine. The parent substance, phthalazine, has been synthesized by a similar reaction from phthaldialdehyde and hydrazine (Gabriel & Pinkus, 1893).

When cyclopaldic acid is oxidized at room temperature with alkaline hydrogen peroxide, oxycyclopaldic acid (XXVI) (6-formyl-5-hydroxy-3-methoxy-4-methylphthalic acid), colourless needles, m.p. 138° (decomp.), is formed in good yield. Unlike cyclopaldic acid, cyclopolic acid is not oxidized with alkaline hydrogen peroxide at room temperature and was recovered unchanged. Oxycyclopaldic acid gives a yellow solution in sodium hydroxide; a purplish red colour with ferric chloride; an immediate yellow precipitate with Brady's reagent; an anhydride (6-formyl-5-hydroxy-3-methoxy-4-methylphthalic anhydride), pale-yellow needles, m.p. 141°; and, on methylation with dimethyl sulphate and anhydrous potassium carbonate in acetone, it gives monomethyl oxycyclopaldate methyl ether (methyl-(2) 6-formyl-3:5-dimethoxy-4-methylphthalate) (XXVII).



(XXVI)



(XXVII)

This mono-ester is soluble in aqueous sodium bicarbonate, gives no colour with sodium hydroxide or ferric chloride, but gives a yellow crystalline precipitate with Brady's reagent. Oxidation of monomethyl oxycyclopaldate methyl ether (XXVII) with alkaline potassium permanganate under mild conditions gives 3-carbomethoxy-4:6-dimethoxy-5-methylphthalic acid, colourless plates, m.p. 135–137° (decomp.), which no longer gives a precipitate with Brady's reagent and on alkaline hydrolysis give the same 4:6-dimethoxy-5-methylbenzene-1:2:3-tricarboxylic acid (VIII) as was obtained by the mild permanganate oxidation of monomethyl cyclopolate methyl ether. Vigorous oxidation of monomethyl oxycyclopaldate methyl ether with hot alkaline potassium permanganate gives the same 4:6-dimethoxybenzene-1:2:3:5-tetracarboxylic acid (IV) as was obtained under the same conditions by the oxidation of monomethyl cyclopolate methyl ether.

The question as to which of the methoxyl groups in cyclopolic acid monomethyl ether (XVI)—whether that in position 6 or that in position 4—is present as a free hydroxyl group in cyclopolic acid itself, and hence also in cyclopaldic acid, resolves itself into a decision whether cyclopolic acid is best regarded as a substituted *ortho*-hydroxycarboxylic acid or as an *ortho*-hydroxyaldehyde. The experimental evidence at present available favours the latter conclusion as shown in structure (XXI) for cyclopolic acid and (XXIIa and b) for cyclopaldic acid, and can be summarized as follows.

(1) Cyclopolic acid gives with aqueous ferric chloride an intense stable purple colour which is almost indistinguishable from that given by both salicylic acid and salicylaldehyde with the same reagent. However, two derivatives of cyclopolic acid have been described in which the CHO group has been modified, namely its Clemmensen reduction product, 5-hydroxy-7-methoxy-4:6-dimethylphthalide (CHO → CH_3) (see p. 613), and its thiosemicarbazone (CHO → CH=N.NH.CS.NH_2). The former derivative gives no ferric colour and the latter only an orange colour. This evidence clearly supports the view that cyclopolic acid is an *ortho*-hydroxyaldehyde, since, if it were an *ortho*-hydroxycarboxylic acid its ferric reaction should not be profoundly affected by modification of the CHO group in another part of the molecule.

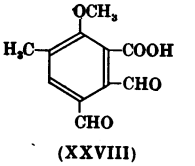
(2) The formation of a substituted coumarin (XXIV*b*) from cyclopaldic acid by heating with acetic anhydride and sodium acetate is an example of the well known Perkin synthesis of coumarins, the simplest example of which is the formation, by the same process, of coumarin itself from salicylaldehyde (*ortho*-hydroxybenzaldehyde).

(3) Most *ortho*-hydroxyaldehydes dissolve in cold sodium hydroxide with the formation of yellow solutions. Cyclopaldic acid and cyclopolide give this reaction although, curiously enough, cyclopolic acid forms a colourless solution.

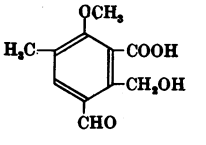
(4) 2:6-Dichloroquinone chloroimide quickly reacts with a solution of cyclopolic acid in buffer (pH 9·2) to give a pure blue colour, while the yellow solution of cyclopaldic acid in the same buffer turns more slowly blue-green with the same reagent. According to Gibbs (1927) and Davidson, Keane & Nolan (1943) this test is specific for phenols with the para position free or occupied by the carboxyl group. The reaction with 2:6-dichloroquinone chloroimide has been used previously in this laboratory in connexion with similar structural problems in the cases of geodin and erdin (Calam, Clutterbuck, Oxford & Raistrick, 1947) and of mycophenolic acid (Birkinshaw, Bracken, Morgan & Raistrick, 1948).

In addition to cyclopolic acid and cyclopaldic acid two other mould metabolic products are known in which all six positions in the benzene ring are substituted. Citrinin from *P. citrinum* Thom (Hetherington & Raistrick, 1931; Coyne, Raistrick & Robinson, 1931), from *Aspergillus terreus* Thom (Raistrick & Smith, 1935), and a number of other moulds, has been synthesized by Cartwright, Robertson & Whalley (1949). Mycophenolic acid, first isolated by Alsberg & Black in 1913 from culture filtrates of *P. stoloniferum* Thom, has more recently been reported from a large number of other species and strains in the *P. brevi-compactum* Dierckx series (Clutterbuck, Oxford, Raistrick & Smith, 1932; Clutterbuck & Raistrick, 1933; Birkinshaw *et al.* 1948; Birkinshaw *et al.* 1952). The close interrelationships in structure between cyclopolic acid, cyclopaldic acid, citrinin and mycophenolic acid have been discussed in detail by Raistrick (1950).

Cyclopaldic acid has a very pronounced fungistatic potency. When tested against the conidia of *Botrytis allii* Munn. in Czapek-Dox medium at pH 3·5 by the technique described by Brian & Hemming (1945) it produced 95–100% inhibition of germination at a concentration of 2·5μg./ml. We found that gladiolic acid, a metabolic product of *P. gladioli* Machacek, described by Brian, Curtis & Hemming (1948), tested at the same time and under the same conditions, gave the same inhibition at a concentration of 10·0μg./ml., a figure which is in good agreement with that previously recorded for gladiolic acid by its describers, i.e. 7·8μg./ml. On the other hand, cyclopolic acid had no effect on the germination of *B. allii* spores at a concentration of 160μg./ml. and gave only a 10% inhibition at a concentration of 320μg./ml., while dihydrogladiolic acid gave no inhibition at 320μg./ml. These findings are of particular interest in view of the close structural relationship between gladiolic acid, structure (XXVIII) established by Raistrick & Ross (1952) and independently by Grove (1952), and cyclopaldic acid, which is clearly 4-hydroxygladiolic acid, and also between dihydrogladiolic acid, structure (XXIX) (Raistrick & Ross, 1952), and cyclopolic acid, which is 4-hydroxydihydrogladiolic acid.

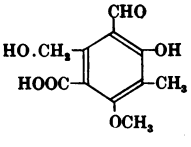


(XXVIII)
Gladiolic acid

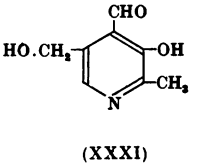


(XXIX)
Dihydrogladiolic acid

There is also a striking structural resemblance, which may be of some biological significance, between cyclopolic acid (XXX) and members of the vitamin B_6 group, in particular pyridoxal (XXXI).



(XXX)



(XXXI)

EXPERIMENTAL

All melting points are uncorrected. Determinations of methoxyl and equivalent by titration were carried out in this department. All other micro-analyses were carried out by Weiler and Strauss, Oxford.

Table 1. *Production of cyclopolic and cyclopaldic acids by strain no.* 85

Incubation period days	Glucose (by polarimeter) (%)	pH	Total ethyl acetate extract (g./flask)	Cyclopolic acid (mg./flask)	Cyclopaldic acid (mg./flask)
6	2·57	3·0	0·18	22	5
9	1·15	3·0	0·30	100	15
13	0·35	3·3	0·36	145	46
16	0·16	3·7	0·31	142	30
20	—	5·4	0·27	110	—
22	—	5·9	0·27	44	24

History of cultures

Two mould cultures were used during this work. Their history is as follows.

P. cyclopium Westling var. *album* G. Smith, L.S.H.T.M. cat. no. 85, was isolated from a mouldy banana skin in May 1947. It was identified and a full description of it was given by our colleague, Mr G. Smith (see Smith, 1951). It is morphologically a typical strain of *P. cyclopium* in all respects except that it is almost pure white in colour and there is an almost total lack of green conidial pigment.

P. cyclopium Westling, L.S.H.T.M. cat. no. 77, was isolated from a mouldy cooked bone in April 1947. It was identified by Mr G. Smith as a typical green-sporing strain of *P. cyclopium* Westling.

Both cultures were isolated originally by Mrs S. Marcus of this department.

Cultural conditions

Quantities of 350 ml. of Raulin-Thom solution (glucose, 75·0 g.; tartaric acid, 4·0 g.; ammonium tartrate, 4·0 g.; $(NH_4)_2HPO_4$, 0·6 g.; $(NH_4)_2SO_4$, 0·25 g.; K_2CO_3, 0·6 g.; $MgCO_3$, 0·4 g.; $FeSO_4.7H_2O$, 0·07 g.; $ZnSO_4.7H_2O$, 0·07 g.; distilled water, 1500 ml.) were distributed in a number of 1 l. conical flasks, plugged with cotton wool, and sterilized. Batches of fifty flasks were inoculated with a spore suspension in sterile distilled water of either strain no. 85 or no. 77 of *P. cyclopium* which had been cultivated on malt-agar slopes at 24° for 7 days, four flasks being inoculated from each malt-agar slope. The inoculated flasks were incubated at 24° in the dark.

Strain no. 85. Growth of this strain was good and after 2 days had extended over the whole surface of the medium. After 5 days the mycelium became convoluted, the upper surface being white to cream coloured, the lower surface yellow to light brown, and wherever it was exposed to the air it slowly turned brown and finally black after 21 days. Otherwise there was little change in the general appearance of the mycelium from 5 days onwards. The culture fluid was pale yellow after 7 days, brownish yellow after 14 days and dark brown after 21 days. It gave a strong brownish-red colour with aqueous $FeCl_3$ from 13 days' incubation onwards.

Strain no. 77. Growth of this strain was good. After 7 days the mycelium was very convoluted, the upper surface being mottled green, the reverse being buff coloured. After 14 and 21 days the only marked change in appearance was that the upper surface was much darker green in colour and the reverse was also darker in colour with some brick coloured areas. The culture fluid was pale yellow after 7 days, yellow after 14 days and brown after 21 days. It gave a strong clear reddish-brown to ruby colour with aqueous $FeCl_3$ after 14 days' incubation.

Neither culture fluid inhibited at a dilution of 1:10 the growth of the Oxford H strain of *Staphylococcus aureus* or the National Collection of Type Cultures strain no. 86 of *Bacterium coli* (*Escherichia coli*) at any period of growth up to 14 days.

Course of metabolism of strain no. 85

A number of flasks from a batch inoculated with strain no. 85 were harvested at frequent intervals. The course of the metabolism was followed by measuring the residual glucose polarimetrically and the approximate yields of cyclopolic and cyclopaldic acids by the isolation methods described below. The results obtained are summarized in Table 1.

It will be seen that the yields of cyclopolic and cyclopaldic acids reach a maximum after 13–16 days' incubation and thereafter decrease quickly. For this reason, in the bulk preparation of cyclopolic and cyclopaldic acids, all flasks of strain no. 85 were harvested after 14 days' incubation at 24°.

Isolation of cyclopolic and cyclopaldic acids

The culture fluid from each batch of fifty 1 l. flasks of either strain no. 85 or strain no. 77 was separated from the mycelium by straining through muslin and was then clarified by filtration after the addition of about 1 g./l. of kieselguhr.

(i) *Strain no.* 85. The clear filtrate (15 l.) after 14 days' growth at 24° was divided into two portions and extracted three times, without acidification, in two 10 l. bottles with ethyl acetate (2 l. each time). After the third extraction the culture filtrate gave only a slight ferric reaction. The combined filtered ethyl acetate extracts (12 l.) were evaporated at about 110 mm. pressure at 40–50° to a residual volume of 200–300 ml. when a quantity of white to cream solid had already separated. The mixture was held at room temperature for some days when the solid material was separated by filtration, washed with a little cold ethyl acetate, and dried. The dried solid consisted of fairly pure cyclopolic acid, the melting point varying in different batches from 139 to 147° (decomp.) (see Table 2, column 4, 1st crop).

Further evaporation of the ethyl acetate mother liquors, usually from two or more batches combined, gave a second crop of less pure cyclopolic acid (see Table 2, column 5, 2nd crop).

The final ethyl acetate mother liquors were evaporated to dryness under reduced pressure giving a partly solid, partly gummy residue which was triturated with cold ether (100–150 ml.) until all the gum had dissolved. The solid was separated by filtration, washed with ether and dried. It consisted essentially of cyclopaldic acid, the melting point of which varied between 183 and 212° (see Table 2, column 6).

Table 2. *Yields of crude cyclopolic and cyclopaldic acids isolated from batches of fifty flasks of strain no.* 85 *grown for* 14 *days at* 24°

Batch no.	Glucose (by polarimeter) (%)	pH	Cyclopolic acid 1st crop (g.)	Cyclopolic acid 1st crop M.p. (decomp.) (°)	Cyclopolic acid 2nd crop (g.)	Cyclopolic acid 2nd crop M.p. (decomp.) (°)	Cyclopaldic acid (g.)	Cyclopaldic acid M.p. (°)
1	0·21	3·3	5·8	143–147	—	—	4·5	183–195
2	0·17	3·5	5·5	141–142				
3	0·32	3·4						
4	0·09	3·5	10·2	147	9·5	130–137	4·0	209–212
5	0·12	3·7						
6	0·13	3·7	3·6	139–140				
7	0·08	3·8	—	—	—	—	3·4	193–212

Table 3. *Production of ether extractable metabolic products by strain no.* 77

Batch no.	Glucose (by polarimeter) (%)	pH	Crop I (g.)	Crop I M.p. (°)	Crop II (g.)	Crop II M.p. (°)	Crop III (g.)	Crop III M.p. (°)	Uncrystallizable ether residue (g.)
1	0·38	3·6	2·49	148–151 (decomp.)	0·61	150–240	—	—	3·3
2	0·36	3·6	4·06	142–145 (decomp.)	1·40	140–175	—	—	3·19
3	0·17	3·6	4·40	142–144 (decomp.)	0·80	172–174	0·08	180–200	3·45
4	0·17	3·6	4·20	138–142 (decomp.)	0·75	132–140	—	—	3·5

Evaporation of the ether mother liquors gave a considerable quantity (41 g. from 350 flasks) of a brown semi-solid, partly crystalline gum from which no other pure crystalline substance could be isolated.

The approximate yields of cyclopolic and cyclopaldic acids obtained from batches of fifty flasks of strain no. 85, incubated for 14 days at 24°, are shown in Table 2.

It will be seen from Table 2 that from a total of 350 flasks 34·6 g. of cyclopolic acid (equal to 4·9 g./fifty flasks) and 11·9 g. of cyclopaldic acid (equal to 1·7 g./fifty flasks) were isolated.

The metabolism solution, which, in batches 1–4 was light yellow in colour, became darker in batches 5–6, and in batch 7 was deep brown in colour. This deepening in colour follows a steady rise in pH from batch 1 to batch 7, and is clearly connected with the decreased yield of cyclopolic acid in batch 6 and its absence from batch 7.

(ii) *Strain no.* 77. The clear culture filtrate (15 l.), after 14 days' growth at 24°, was acidified with 5 N-HCl (150 ml.). The acidified filtrate was divided into two equal portions and each portion was extracted three times with ether (2 l. + 2 l. + 1·5 l.), centrifuging being necessary to obtain good separation. The extracted filtrate now gave only a brownish ferric colour. The combined ether extracts were evaporated. Crystalline material began to separate after evaporating to about 400 ml. and evaporation was continued to about 150 ml. The concentrate and crystals were held at 0° for 3 days and filtered, giving crop I. Crops II and III were obtained by further concentration of the mother liquor. On drying the final ether mother liquor there remained a dark-red oily residue from which no further crystalline material could be obtained. The yields of extracted metabolic products from batches of fifty flasks are given in Table 3.

The crude extracted metabolic products (crops I–III) consisted of a mixture of cyclopolic acid and cyclopaldic acid which proved much more difficult to separate in a state of purity than the ethyl acetate extracts from strain no. 85. The simplest method of fractionation was as follows. Crop I from batch 4 (4·20 g.; m.p. 138–142°) was well stirred with water (100 ml.) at 50° and the undissolved portion was separated by filtration. It was then dissolved in boiling water and filtered from a trace of dark-brown material. On cooling the filtrate, brownish crystals (0·37 g.) separated, m.p. 210–213°, raised to 224° on further purification and not depressed on admixture with cyclopaldic acid from strain no. 85.

Two crops of colourless crystals (0·91 g., m.p. 140–144° (decomp.); 0·74 g., m.p. 142–147° (decomp.)) were obtained on cooling the aqueous filtrate obtained at 50°. Similarly, three crops of crystals were obtained on evaporation of the aqueous mother liquors from the crude cyclopaldic acid, giving in all a total of 2·84 g. of crude cyclopolic acid.

Direct comparison of strains no. 85 *and* 77

(By Mr M. M. Abou Zeid)

A direct comparison of the yields of cyclopolic and cyclopaldic acids produced by strains nos. 85 and 77 was made some 2 years after the completion of the work already described. The same medium and cultural conditions were used but the metabolic products were isolated in each case by the ethyl acetate extraction method described on p. 617. A summary of the results obtained follows.

Strain no. 85. Fifty flasks; incubation period, 14 days; pH, 3·1; residual glucose by polarimeter, 0·44%; weight of dry mycelium, 188 g.; crude cyclopolic acid obtained, 3·12 g., m.p. 147–148° (decomp.); crude cyclopaldic acid obtained, 1·84 g., m.p. 207–209°, which on crystallization from boiling water (300 ml./g. + charcoal) gave cyclopaldic acid as colourless needles, 0·96 g., m.p. 223–224°; uncrystallizable residue from ether mother liquors, 6·55 g.

Strain no. 77. Fifty flasks; incubation period, 14 days; pH, 3·3; residual glucose by polarimeter, 0·46%; weight of dry mycelium, 209 g.; crude cyclopolic acid obtained, 12·4 g., m.p. 147–148° (decomp.); no cyclopaldic acid could be isolated; uncrystallizable residue from ether mother liquors, 9·02 g.

Cyclopolic acid

Purification. Crude cyclopolic acid (11·3 g.) was recrystallized by solution in boiling ethyl acetate (0·5 l.) and the addition of benzene (1·5 l.). Yield of pure cyclopolic acid 7·0 g.

General properties. Cyclopolic acid crystallizes in colourless plates, m.p. 147–148° (decomp.). When finely ground it melts at 138–140° (decomp.). The acid melts with effervescence and loss of water to a clear straw-coloured liquid which does not reset on cooling. (Found: C, 55·0; H, 5·1; OCH_3, 12·9; *C*-CH_3, 4·5 = 0·71 *C*-CH_3 groups; equivalents, by conductometric titration, 236, 120. $C_{11}H_{12}O_6$ requires C, 55·0; H, 5·0; $1OCH_3$, 12·9; $1C$-CH_3, 6·3%; equivalent, titrating as a monobasic acid, 240; as a dibasic acid, 120.) The acid (*c*, 2·5 in methanol) showed no optical activity in a 2 dm. tube. It is soluble in water, methanol, ethyl acetate, ether and acetone, but is almost insoluble in benzene and light petroleum. It dissolves at once with effervescence in cold aqueous $NaHCO_3$ to a colourless solution. Its solution in cold 2N-NaOH is also colourless. Its aqueous solution gives an intense stable purple colour, almost indistinguishable from that given by salicylic acid, with aqueous $FeCl_3$. Its ethanolic solution gives a similar colour, but somewhat bluer in shade, with ethanolic $FeCl_3$. Brady's reagent (0·32% 2:4-dinitrophenylhydrazine in aqueous 2N-HCl) quickly gives a deep-yellow crystalline precipitate. It reduces ammoniacal $AgNO_3$ on warming, but it does not reduce Fehling's solution on heating for several minutes. It gives a negative reaction with Schiff's reagent. Under the conditions described for the test on cyclopaldic acid (see p. 624) no colour is obtained with conc. NH_3 (*d*, 0·880). With bleaching powder solution it gives a yellow colour fading to colourless in a few minutes. It gives with bromine water a turbidity soluble in excess of the reagent. When dissolved in buffer solution, pH 9·2, and treated with a few drops of an aqueous suspension of 2:6-dichloroquinone chloroimide a deep-blue colour is formed in 2 min. which is stable for 3 days.

Functional derivatives

Cyclopolic acid mono-2:4-dinitrophenylhydrazone. Brady's reagent (40 ml.) was added to a cold solution of cyclopolic acid (100 mg.) in water (10 ml.). The resulting deep-yellow precipitate of microprisms was separated by filtration, washed and dried (weight 113 mg.), m.p. 190·5–191° (decomp.) not changed on recrystallization from ethanol. (Found: C, 48·5; H, 3·6; N, 13·1. $C_{17}H_{16}O_9N_4$ requires C, 48·6; H, 3·8; N, 13·3%.) *Cyclopolic acid mono-2:4-dinitrophenylhydrazone* gave a red colour with ethanolic NaOH (Neuberg reaction) confirming a mono-derivative.

Cyclopolic acid thiosemicarbazone. A solution of cyclopolic acid (120 mg.) in methanol (1·5 ml.) was added to a hot solution of thiosemicarbazide (45 mg.) and anhydrous sodium acetate (60 mg.) in 0·25N-HCl (2 ml.), and the whole was heated at 100° for 5 min. It was then cooled, diluted with water (40 ml.) and held at room temperature for 3 days. The hard slightly brown solid which separated was collected (weight 89 mg.), m.p. 180·5–182° (decomp.). Recrystallization from ethanol (+ charcoal) yielded colourless microprisms of the same melting point. (Found: C, 45·9; H, 5·0; N, 13·6; S, 10·3. $C_{12}H_{15}O_5N_3S$ requires C, 46·0; H, 4·8; N, 13·4; S, 10·2%.) *Cyclopolic acid thiosemicarbazone* is readily soluble in cold aqueous $NaHCO_3$. Its ethanolic solution gives an orange colour with ethanolic $FeCl_3$.

Acetylation of cyclopolic acid. Formation of cyclopolide triacetate. A mixture of cyclopolic acid (0·50 g.), sodium acetate (1·0 g.) and acetic anhydride (2 ml.) was refluxed for 0·5 hr. On adding water a light-brown solid (0·71 g.), m.p. 110–118° separated. Two crystallizations from 60% aqueous methanol gave colourless needles (0·32 g.), m.p. 117°. (Found: C, 55·8; H, 4·8; OCH_3, 8·4; $COCH_3$, 33·8. $C_{17}H_{18}O_9$ requires C, 55·7; H, 5·0; $1OCH_3$, 8·5; $3COCH_3$, 35·2%.) *Cyclopolide triacetate* is insoluble in cold 2N-NaOH except on long standing. Its ethanolic solution gives no colour with ethanolic $FeCl_3$ nor any precipitate with Brady's reagent. When this acetylation was carried out at 180–190° for 6·5 hr. the product was still cyclopolide triacetate. Under these conditions cyclopaldic acid gave a substituted coumarin (see p. 624).

A solution of cyclopolic acid (0·50 g.) in pyridine (5 ml.) and acetic anhydride (5 ml.) was held at 37° for 4 days. The solid formed (0·71 g.), m.p. 112–113°, on the addition of water, was collected and crystallized from aqueous methanol yielding thick colourless needles (0·41 g.), m.p. 116·5–117°, not depressed on admixture with cyclopolide triacetate prepared with sodium acetate and acetic anhydride.

Cyclopolide. (*a*) From cyclopolide triacetate. Cyclopolide triacetate (0·40 g.) was refluxed with aqueous 2N-H_2SO_4 (20 ml.) for 4 hr. The resulting light-brown solid (0·35 g.), m.p. 163–165°, was collected and crystallized twice from aqueous 66% ethanol giving colourless delicate needles, m.p. 169°, which reset and remelted at the same temperature. Sublimation in a high vacuum at 120–140° gave fluffy needles of the same melting point. (Found: C, 59·6, 59·5; H, 4·6, 4·6; OCH_3, 14·0; *C*-CH_3, 6·8; mol.wt. (Rast) 232. $C_{11}H_{10}O_5$ requires C, 59·4; H, 4·5; $1OCH_3$, 14·0; $1C$-CH_3, 6·8%; mol.wt. 222.) The trivial name *cyclopolide* is given to this substance despite the fact that it is not strictly accurate nomenclature. Cyclopolide is almost insoluble in cold water, but dissolves readily in cold aqueous $NaHCO_3$. It dissolves in cold 2N-NaOH to a pale-yellow solution which deepens in colour on warming. Its ethanolic solution gives a ruby-red colour with ethanolic $FeCl_3$, very similar to that given by cyclopaldic acid in ethanol, and an immediate orange precipitate with Brady's reagent. When cyclopolide is warmed with ammoniacal $AgNO_3$ a black precipitate is slowly formed but without the formation of a distinct silver mirror.

Cyclopolide was also obtained, though in much smaller yields, by each of the following methods.

(*b*) Cyclopolide triacetate (2·8 g.) was refluxed for 2 hr. with 2N-HCl (20 ml.) and methanol (40 ml.). Fractionation, from ether and aqueous methanol, of the brown gum remaining after removal of the solvent *in vacuo* at 50° yielded cyclopolide (0·37 g.), m.p. 161–164°.

(*c*) A solution of cyclopolic acid (0·20 g.) in 2N-H_2SO_4 (17 ml.) was refluxed for 3·5 hr. A light-brown solid (0·15 g.) separated. On fractional crystallization from aqueous ethanol (+ charcoal) it yielded needles (30 mg.), m.p. 152°, which were purified by fractional sublimation in a high vacuum giving cyclopolide (11 mg.), m.p. 164–165°.

(*d*) Cyclopolic acid (0·20 g.) was heated at 155° for 20 min. and then at 170° for 2 min. Fractional sublimation of the melt in a high vacuum gave at 120–140° 4 mg. of cyclopolide, m.p. 165°. The melting point of the specimens of cyclopolide from methods (*b*), (*c*) or (*d*) was not depressed on admixture with pure cyclopolide, m.p. 169° from method (*a*).

Derivatives of cyclopolide. (*a*) *Cyclopolide monomethyl ether.* An excess of ethereal diazomethane was added to a

solution of cyclopolide (94 mg.) in acetone (5 ml.). After standing overnight the solution was filtered and evaporated to dryness. The residual slightly gummy solid (102 mg.) was crystallized twice from aqueous methanol giving *cyclopolide monomethyl ether* as long colourless needles (70 mg.), m.p. 169–170°, resetting and remelting at the same temperature. (Found: C, 61·1, 61·0; H, 5·1, 5·1; OCH_3, 26·1. $C_{12}H_{12}O_5$ requires C, 61·0; H, 5·1; $2OCH_3$, 26·3%.) Cyclopolide monomethyl ether is insoluble in aqueous $NaHCO_3$ and does not dissolve in cold 2N-NaOH except on long standing. It gives no colour with $FeCl_3$ in ethanolic solution.

(*b*) *Mono-2:4-dinitrophenylhydrazone of cyclopolide monomethyl ether.* Brady's reagent (40 ml.) was added to a hot solution of cyclopolide monomethyl ether (48 mg.) in ethanol (8 ml.). The resulting orange gelatinous precipitate (76 mg.) was collected and crystallized from pyridine giving *mono-2:4-dinitrophenylhydrazone of cyclopolide monomethyl ether* as silky deep-orange needles (65 mg.) which on heating decompose progressively from 280 to 360° without melting. (Found: C, 52·2; H, 3·8; N, 13·5. $C_{18}H_{16}O_8N_4$ requires C, 51·9; H, 3·9; N, 13·5%.)

(*c*) *Diacetate of cyclopolide monomethyl ether.* The crude product (110 mg.) resulting from the alkaline (2N-KOH) hydrolysis of the methyl ether of methyl cyclopolate (320 mg.) was refluxed for 0·5 hr. with anhydrous sodium acetate (70 mg.) and acetic anhydride (0·3 ml.). The light-brown solid (120 mg.), formed on the addition of water, was collected, crystallized twice from light petroleum, b.p. 60–80°, giving crystals (26 mg.), m.p. 128–129°, and then twice from aqueous methanol giving the *diacetate of cyclopolide monomethyl ether* as colourless blades (12 mg.), m.p. 133·5–134°. (Found: C, 57·2; H, 5·5; OCH_3, 18·6. $C_{16}H_{18}O_8$ requires C, 56·8; H, 5·4; $2OCH_3$, 18·3%.) Its ethanolic solution gives no precipitate with Brady's reagent. The diacetate of cyclopolide monomethyl ether (5 mg.) was refluxed for 2 hr. with 2N-H_2SO_4 (1 ml.). On cooling, light-brown needles (2·5 mg.) separated, m.p. 159° not depressed on admixture with authentic cyclopolide monomethyl ether.

Methylation of cyclopolic acid with diazomethane

Formation of methyl cyclopolate monomethyl ether. An excess of ethereal diazomethane was added to a solution of cyclopolic acid (0·50 g.) in acetone (10 ml.) giving an immediate strong effervescence. After standing overnight the solution was filtered and evaporated to dryness giving a pale-amber gum (0·55 g.) which partially solidified on standing. The crystalline portion was separated from insoluble gum by extraction with light petroleum, b.p. 40–60°, which, on evaporation, gave small plates (0·22 g.), m.p. 80°. This material was crystallized from water giving *methyl cyclopolate monomethyl ether* (0·13 g.) as colourless plates, m.p. 87·5–88°. (Found: C, 58·3, 58·4; H, 6·0, 5·9; OCH_3, 34·4; active H atoms (in anisole), 0·63 atom. $C_{13}H_{16}O_6$ requires C, 58·2; H, 6·0; $3OCH_3$, 34·7%.) An aqueous solution of the ester gives no colour with $FeCl_3$, but quickly gives a yellow precipitate with Brady's reagent.

In the hope of obtaining cyclopolic acid monomethyl ether, the total crude ester (0·59 g.), resulting from a second methylation of cyclopolic acid (0·50 g.) with ethereal diazomethane, was dissolved in methanol (5 ml.) and treated with 5 ml. of methanolic potash (2 g. KOH in methanol 10 ml.). After holding for 4 days at room temperature water (10 ml.) and 2N-HCl (8 ml.) were added. Methanol was removed *in vacuo* and the aqueous solution was extracted with ether. On removal of the solvent the residue slowly crystallized (0·52 g.). This product could not be satisfactorily purified. It was characterized, however, by conversion into a mono-2:4-dinitrophenylhydrazone which was obtained in good yield.

Mono-2:4-dinitrophenylhydrazone of cyclopolic acid monomethyl ether. Brady's reagent (10 ml.) was added to a solution in water (10 ml.) of the above crude cyclopolic acid monomethyl ether (20 mg.). The yellow crystalline precipitate (25 mg.), m.p. 287° (decomp.), which readily formed, was collected and crystallized from methanol giving the *mono-2:4-dinitrophenylhydrazone of cyclopolic acid monomethyl ether* as yellow needles, m.p. 287° (decomp.). (Found (*a*) on sample dried at room temperature in a high vacuum: C, 47·9; H, 4·5; N, 12·8. $C_{18}H_{18}O_9N_4.1H_2O$ requires C, 47·8; H, 4·5; N, 12·4%; (*b*) on sample dried at 100° *in vacuo*, loss in weight 3·7; $C_{18}H_{18}O_9N_4.1H_2O$ requires H_2O, 4·0%; C, 49·9; H, 4·4; N, 12·9. $C_{18}H_{18}O_9N_4$ requires C, 49·8; H, 4·2; N, 12·9%.) Both the hydrated and anhydrous forms are soluble in cold saturated aqueous $NaHCO_3$ and give a red Neuberg reaction confirmatory of a mono-2:4-dinitrophenylhydrazone.

Conversion of cyclopolic acid into cyclopaldic acid

Cyclopolic acid, m.p. 147° (decomp.), (0·10 g.), potassium periodate (0·10 g.) and N-H_2SO_4 (10 ml.) were boiled under reflux for 5 min. giving a pale-brown solution which then clouded with some decomposition. On cooling, needles (36 mg.), m.p. 215–219°, separated. Sublimation in a high vacuum at 150–160° gave colourless crystals of cyclopaldic acid (26 mg.), m.p. 222–223° with sublimation, unchanged on mixing with cyclopaldic acid, m.p. 224–225°, isolated directly from the culture filtrate of *P. cyclopium* var. *album* no. 85. The mixture reset on cooling and remelted at the same temperature.

Degradation products of cyclopolic acid

A. *Clemmensen reduction of cyclopolic acid and methylation of reduction product. Isolation of 5:7-dimethoxy-4:6-dimethylphthalide (XIV)*

Granulated zinc (20 g.) was immersed in an aqueous solution of $HgCl_2$ (40 ml. of a 5% solution) for 1 hr. Part of the washed amalgam (12 g.) was suspended in conc. HCl (15 ml.) and heated under reflux. Cyclopolic acid (0·50 g.), dissolved in hot water (15 ml.), was gradually added during 0·75 hr., and during the reduction two additions of 5 ml. of conc. HCl were made at hourly intervals. The course of the reduction was followed by periodic addition of Brady's reagent to a few drops of the reaction liquid which gave at first an orange, and later a yellow precipitate. After 3·5 hr. no precipitate was obtained with Brady's reagent and the reduction was stopped. The reaction mixture was held overnight, when the gummy reduction product had solidified and a further quantity of colourless solid had separated. The total product was mechanically separated from the residual Zn, filtered, washed with water and dried, wt. 0·36 g., m.p. 193–199°. Since no satisfactory solvent could be found for the crystallization of the crude reduction product the whole of it was fractionally sublimed in a high vacuum, and at 160–170° gave a colourless solid (60 mg.), m.p. 202–203°, which, on crystallization from benzene, gave *5-hydroxy-7-methoxy-4:6-dimethylphthalide* as colourless lustrous needles,

m.p. 202·5–203·5°, resetting and remelting at the same temperature. (Found: C, 63·7; H, 6·1; OCH_3, 14·9. $C_{11}H_{12}O_4$ requires C, 63·5; H, 5·8; $1OCH_3$, 14·9%.) It is soluble in cold aqueous 2N-Na_2CO_3 and its methanolic solution gives no colour with $FeCl_3$. The reduction product (25 mg.) was methylated in the usual way with dimethyl sulphate, acetone and anhydrous K_2CO_3. The crude methylation product (22 mg.), m.p. 98–99°, was crystallized from light petroleum, b.p. 60–80°, giving colourless needles (15 mg.), of *5:7-dimethoxy-4:6-dimethylphthalide*, m.p. 101·5–102°, resetting and remelting at the same temperature and not depressed on admixture with an authentic synthetic specimen, m.p. 98–100° (see p. 627). (Found: C, 64·7; H, 6·3; OCH_3, 28·1. $C_{12}H_{14}O_4$ requires C, 64·9; H, 6·4; $2OCH_3$, 27·9%.) It does not dissolve in cold 2N-NaOH.

B. *Potash fusion of cyclopolic acid at* 300°. *Formation of* 3:5-*dihydroxy*-4-*methylbenzoic acid*

KOH (2·5 g.) and water (0·5 ml.) were heated to 150° in a nickel crucible floating on a Wood's metal bath. Cyclopolic acid (0·50 g.) was added, and during 15 min. the bath temperature was raised to 300° and maintained at this temperature for a further 10 min. when the original vigorous reaction had practically ceased. The dark-brown fluid melt was cooled, water (50 ml.) was added and the resultant solution was adjusted to pH 2 with 2N-HCl. An aqueous solution of $CaCl_2$ (0·2 g.) was added. After standing overnight the small precipitate of calcium oxalate was filtered off and the filtrate was extracted with ether. On removal of the solvent there remained a pale-brown resinous solid (0·33 g.) which was purified by fractional sublimation in a high vacuum, giving at 150–180° a small gummy upper layer which was not identified and a light-brown solid lower layer (0·18 g.), m.p. 254–259° (decomp.). This lower layer was purified by repeated crystallization from water giving colourless needles of 3:5-dihydroxy-4-methylbenzoic acid (3:5-dihydroxy-*p*-toluic acid), m.p. 269–270° (decomp.) not depressed on admixture with an authentic synthetic specimen (see p. 626). (Found (*a*) on sample dried to constant weight at room temperature: C, 53·9; H, 5·4; H_2O, 5·2, 5·4; OCH_3, nil. Calc. for $C_8H_8O_4 \cdot \frac{1}{2}H_2O$: C, 54·2; H, 5·1; H_2O, 5·1%. (*b*) On sample dried to constant weight at 100°: C, 57·4, 57·2; H, 4·7, 4·8; equivalent by titration 170. Calc. for $C_8H_8O_4$: C, 57·2; H, 4·8%; equiv., titrating as a monobasic acid, 168.) An aqueous solution of the acid gives no colour with $FeCl_3$.

The above acid (0·38 g.) was esterified with an excess of diazomethane in ether in the usual way. The resultant light-yellow solid (0·42 g.) was crystallized first from ethyl acetate-light petroleum b.p. 60–80° giving colourless needles (0·22 g.), m.p. 187–189°. Recrystallization from benzene gave colourless needles of pure methyl 3:5-dihydroxy-*p*-toluate (0·11 g.), m.p. 189–190°, not depressed on admixture with an authentic synthetic specimen, m.p. 189–190°. (Found: C, 59·2, 59·4; H, 5·5, 5·5; OCH_3, 17·0. Calc. for $C_9H_{10}O_4$: C, 59·3; H, 5·5; $1OCH_3$, 17·0%.) An aqueous solution of the ester gives a lavender colour with $FeCl_3$.

The above methyl 3:5-dihydroxy-*p*-toluate (125 mg.) was acetylated in the usual way with anhydrous sodium acetate (250 mg.) and acetic anhydride (1 ml.). The acetylation product was purified by crystallization from methanol (+charcoal) and then from light petroleum b.p. 60–80°. *Methyl* 3:5-*diacetoxy-p-toluate* was thus obtained as colourless shining needles (63 mg.), m.p. 101–101·5° not depressed on admixture with an authentic synthetic specimen, m.p. 101–101·5°. (Found: C, 59·0, 58·7; H, 5·4, 5·3; OCH_3, 11·6. $C_{13}H_{14}O_6$ requires C, 58·7; H, 5·3; $1OCH_3$, 11·7%.)

C. iso*Cyclopaldic acid*

Action of KOH *at* 150° *on cyclopolic acid. Isolation of* iso*cyclopaldic acid*

A mixture of cyclopolic acid (0·40 g.), KOH (2 g.) and water (2 ml.), contained in a nickel crucible, was held at 150° in a metal bath. Test samples of the melt were periodically acidified and treated with Brady's reagent until a precipitate ceased to be formed (15 min.). The melt was then cooled, dissolved in water (15 ml.) and acidified with HCl. The resulting light-brown curdy precipitate was collected, dried (0·18 g.), m.p. 190–200° (decomp.), and crystallized to constant melting point from aqueous methanol (+charcoal). iso*Cyclopaldic acid* was thus obtained as colourless needles, m.p. 223·5–224° (decomp.). (Found: loss in weight at 100°, 6·8. $C_{11}H_{10}O_6 \cdot H_2O$ requires H_2O, 7·0%; on dried sample: C, 55·7, 55·9; H, 4·4, 4·2; OCH_3, 13·0. $C_{11}H_{10}O_6$ requires C, 55·5; H, 4·2; $1OCH_3$, 13·0%.) The acid is readily soluble in ether, methanol and ethyl acetate and slightly soluble in benzene and water. Its aqueous solution gives with $FeCl_3$ a deep reddish-purple colour which is much redder in tone than that given by cyclopolic acid.

Monomethyl ether of methyl iso*cyclopaldate.* *iso*Cyclopaldic acid (75 mg.) was methylated in the usual way with dimethyl sulphate (0·45 ml.), acetone (7 ml.) and anhydrous K_2CO_3 (0·40 g.) for 1·5 hr. The recovered crude methylation product (64 mg.), m.p. 136–139°, was crystallized from light petroleum giving the *monomethyl ether of methyl* iso*cyclopaldate* as long colourless silky needles (38 mg.), m.p. 139–139·5° not depressed on admixture with the synthetic *methyl ester of 4-carboxy-5:7-dimethoxy-6-methylphthalide*, m.p. 139–139·5° (see p. 627). (Found: C, 58·6, 58·8; H, 5·1, 5·3; OCH_3, 34·8. $C_{13}H_{14}O_6$ requires C, 58·6; H, 5·3; $3OCH_3$, 35·0%.)

iso*Cyclopaldic acid monomethyl ether.* The monomethyl ether of methyl *iso*cyclopaldate (0·30 g.) was refluxed for 0·75 hr. with a solution of KOH in 1:1 water-ethanol (0·2N; 10 ml.). Ethanol was removed *in vacuo* at 50° and the residue was diluted with water and acidified with HCl. The resulting precipitate was collected (0·27 g.), m.p. 194·5–195·5°, and crystallized from methanol (+charcoal) giving iso*cyclopaldic acid monomethyl ether* as colourless needles, m.p. 198–199° not depressed on admixture with synthetic *4-carboxy-5:7-dimethoxy-6-methylphthalide*, m.p. 198–198·5° (see p. 627). (Found: C, 57·2, 57·2; H, 4·6, 4·7; OCH_3, 24·3. $C_{12}H_{12}O_6$ requires C, 57·2; H, 4·8; $2OCH_3$, 24·6%.) The acid titrates in the cold to phenolphthalein as a monobasic acid, in 1:1 methanol-water solution. (Found: equiv. 249. $C_{12}H_{12}O_6$, titrating as a monobasic acid, requires equiv. 252.) On heating with an excess of 0·1N-NaOH and back titration it titrates as a dibasic acid. (Found: equiv. 125. $C_{12}H_{12}O_6$, titrating as a monoacid monolactone requires equiv. 126.)

Decarboxylation of iso*cyclopaldic acid*

(*a*) *With* HI *and red* P. *Isolation of β-orcinol* (2:6-*dihydroxy*-1:4-*dimethylbenzene*). In a quantitative estimation *iso*-cyclopaldic acid (200 mg.) was refluxed with HI (*d*, 1·7; 3 ml.) and red P (0·3 g.) in a slow stream of CO_2-free N_2. The effluent gases were first scrubbed through a red P-water trap and then through bubblers containing 0·2N-$Ba(OH)_2$ which was titrated with 0·1N-HCl at intervals. CO_2 was evolved as

follows. After 0·75 hr., 0·55 mol. equiv. CO_2; 3 hr., 1·33 mol. equiv.; 7 hr., 1·89 mol. equiv.; 9 hr., 2·06 mol. equiv.

In a preparative experiment *iso*cyclopaldic acid (0·73 g.) was refluxed in N_2 for 20 hr. with HI (*d*, 1·7; 10 ml.) and red P (3 g.). P was removed by filtration, the orange filtrate was treated with aqueous NaOH (40%; 5 ml.) and the still acid solution was extracted with ethyl acetate (4 × 20 ml.). The extract was washed with water and aqueous $Na_2S_2O_3$ and dried over anhydrous Na_2SO_4. Removal of the solvent at 50° *in vacuo* yielded pale-yellow gummy needles (0·39 g.), m.p. 145–155° which were purified to constant melting point by repeated sublimation in a high vacuum and crystallization from benzene, giving finally β-orcinol (108 mg.) as colourless jagged needles, m.p. 161·5–163° not depressed on admixture with authentic synthetic β-orcinol, m.p. 161·5–162·5° (see p. 626). (Found: C, 69·7, 69·8; H, 7·2, 7·4. Calc. for $C_8H_{10}O_2$: C, 69·5; H, 7·3%.) Its aqueous solution gave a blue colour with a faint red tinge with $FeCl_3$.

β-Orcinol (50 mg.), obtained by decarboxylation of *iso*cyclopaldic acid, was acetylated at 140° in the usual way with anhydrous sodium acetate (50 mg.) and acetic anhydride (0·2 ml.). The gum obtained on addition of water was extracted with ether and the ethereal extract was washed with aqueous Na_2CO_3 and water. On removal of the solvent from the dried extract there remained a colourless gum (65 mg.) which slowly crystallized, m.p. 42–43°. Crystallization from light petroleum, b.p. 40–60°, gave β-orcinol diacetate as colourless needles, m.p. 44–45° unchanged on further recrystallization and not depressed on admixture with an authentic synthetic specimen, m.p. 44–44·5° (see p. 626). (Found: C, 65·0, 65·0; H, 6·2, 6·4. Calc. for $C_{12}H_{14}O_4$: C, 64·9; H, 6·4%.)

(*b*) *With copper chromite and quinoline. Methylation of decarboxylation product and isolation of* 5:7-*dimethoxy*-6-*methylphthalide. iso*Cyclopaldic acid (174 mg.) was heated at 180–190° with copper chromite (150 mg.) and pure redistilled quinoline (4 ml.) in a stream of CO_2-free N_2. The effluent gases were first scrubbed through 2 N-H_2SO_4 and then through 0·2 N-$Ba(OH)_2$ which was titrated with 0·1 N-HCl at intervals. CO_2 was evolved as follows. After 0·5 hr., 0·58 mol. equiv.; 2 hr., 0·76 mol. equiv.; 2·25 hr., 0·82 mol. equiv. The reaction was now stopped. 5 N-HCl (20 ml.) was added and the solution was extracted first with ether (4 × 20 ml.) and then with ethyl acetate. The extracts were washed successively with 2 N-HCl, water, aqueous $NaHCO_3$ and water. On removal of the solvent from the dried extracts there remained an oily solid (24 mg.) from ether, and a gummy solid (31 mg.) from ethyl acetate. The combined extracts, which gave a dirty brown-red colour with $FeCl_3$ in methanol, were methylated in the usual way with dimethyl sulphate (0·3 ml.), K_2CO_3 (0·4 g.) and acetone (7 ml.). A methanolic solution of the crude methylated product was left to evaporate to dryness and the resulting brown solid (40 mg.) was separated mechanically from an oily residue. The solid was purified by sublimation *in vacuo* at 140° followed by crystallization from light petroleum, b.p. 60–80°, giving 5:7-*dimethoxy*-6-*methylphthalide* as long colourless lustrous needles (22 mg.), m.p. 171·5–172·5° not depressed on admixture with an authentic synthetic specimen, m.p. 172–172·5° (see p. 627). (Found: C, 63·2; H, 5·7; OCH_3, 29·5. $C_{11}H_{12}O_4$ requires C, 63·5; H, 5·8; $2OCH_3$, 29·8%.)

(*c*) *With aqueous* KOH. *Methylation of decarboxylation product and isolation of* 5:7-*dimethoxy*-6-*methylphthalide. iso*Cyclopaldic acid (0·20 g.) was refluxed for 6·5 hr. with aqueous KOH (28% (w/v); 5 ml.). The solution was acidified with HCl and extracted with ether. The ethereal extract was washed with aqueous $NaHCO_3$ and dried over Na_2SO_4. Acidification of the $NaHCO_3$ extract gave unchanged *iso*cyclopaldic acid (100 mg.). Removal of the solvent from the ether extract yielded an oily red solid (41 mg.) which gave an intense purple colour with methanolic $FeCl_3$. The oily red solid (40 mg.) was methylated with dimethyl sulphate, K_2CO_3 and acetone and gave a small yield (2 mg.) of 5:7-*dimethoxy*-6-*methylphthalide*, m.p. 168–169° not depressed on admixture with an authentic synthetic specimen, m.p. 172–172·5° (see p. 627).

During the preparation of *iso*cyclopaldic acid from cyclopolic acid by heating with KOH at 150° (see p. 621) there accumulated a considerable quantity of acidified aqueous liquors from which the *iso*cyclopaldic acid had been removed by filtration. These liquors, on treatment as described above, gave a sodium bicarbonate-insoluble gum which gave an intense purple colour with $FeCl_3$ and on methylation gave 5:7-dimethoxy-6-methylphthalide.

Degradation of the lichen acid, barbatolic acid, to 5:7-*dimethoxy*-6-*methylphthalide*

Through the courtesy of Dr E. E. Suominen, Chemical Laboratory, University of Helsinki, Finland, we received a specimen of the reduction product, $C_{19}H_{20}O_8$, m.p. 189°, prepared by him by reduction with Zn dust and HI at 70° of the methyl ester of barbatolic acid from the lichen *Alectoria implexa* (Hoffm.) Nyl. f. *fuscidula* Arn. (Suominen, 1939). The reduction product (18 mg.) was refluxed for 4 hr. with dimethyl sulphate (0·4 ml.), dry acetone (10 ml.) and anhydrous K_2CO_3 (0·25 g.). A second portion of K_2CO_3 (0·25 g.) was added during the reaction. Acetone was then removed *in vacuo*, water (10 ml.) was added and the mixture was shaken vigorously for 0·5 hr. The methylated product was extracted with ether (3 × 10 ml.), and on removal of the ether was obtained as a pale-yellow thick gum. This crude product, without further purification, was refluxed for 4 hr. with ethanolic NaOH (0·2 N; 4 ml.). Water was added, the solution was acidified with HCl and the hydrolysis products were extracted with ether (4 × 10 ml.). The ethereal extract was washed successively with water, saturated $NaHCO_3$ solution and again with water. On removal of the solvent from the dried (Na_2SO_4) extract there remained colourless gummy needles (5 mg.), m.p. 156–161°. These were crystallized from aqueous methanol giving long colourless needles, m.p. 171·5°, resetting and remelting at the same temperature and not depressed on admixture with synthetic 5:7-*dimethoxy*-6-*methylphthalide*, m.p. 172–172·5° (see p. 627). The mixed melt reset and remelted at the same temperature. Suominen (1939), whose method for the preparation of this substance we followed, except that he methylated the reduction product, m.p. 189°, with diazomethane for 2 months, gives the m.p. as 173·5°.

D. $KMnO_4$ *oxidation experiments*

(*a*) *Oxidation of methyl cyclopolate monomethyl ether with cold alkaline* $KMnO_4$. *Isolation of* 4:6-*dimethoxy*-5-*methylbenzene*-1:2:3-*tricarboxylic acid* (*VIII*). The monomethyl ether of methyl cyclopolate (1·2 g.) was dissolved in cold aqueous 0·04 N-KOH (24 ml.), and aqueous $KMnO_4$ (5%; 50 ml. equiv. to 5·3 atoms O) was added. After standing at

room temperature for 24 hr. almost all the $KMnO_4$ had been utilized. A further 12 ml. of the same $KMnO_4$ solution equiv. to 1·3 atoms O were therefore added. The solution was kept for a further 18 hr., when there was much residual $KMnO_4$. The precipitated MnO_2 was separated by filtration and washed with water. The combined filtrate and washings were decolorized with Na_2SO_3 and evaporated *in vacuo* to 15 ml. The concentrate was acidified with HCl and extracted with ethyl acetate (5 × 20 ml.). Removal of the solvent from the washed and dried (Na_2SO_4) extracts yielded a colourless gum (0·55 g.). The gum was refluxed with ether (10 ml.) giving a colourless solid which was washed with cold ether (10 ml.). The solid residue (0·23 g.), m.p. 185–190°, was collected and was purified by crystallization from ether giving colourless compact prisms (0·13 g.). (Found, on material dried to constant weight at 100° *in vacuo*: C, 54·3, 54·2; H, 3·9, 4·0; OCH_3, 23·4; equiv. by titration 88. $C_{12}H_{10}O_7$, i.e. $C_{12}H_{12}O_8 - H_2O$, requires C, 54·1; H, 3·8; $2OCH_3$, 23·3%; equiv., titrating as a tribasic acid (mono-acid monoanhydride), 89.) 4:6-*Dimethoxy-5-methylbenzene*-1:2:3-*tricarboxylic acid* behaves in a characteristic fashion on heating, with the formation of the anhydride (IX). If placed in a bath, previously heated to 150°, it rapidly melts with vigorous effervescence (loss of water), but resets immediately and then remelts at 195°. If heated slowly from room temperature it loses its lustre, but no effervescence occurs. It melts at 198·5–199·5°, resets on cooling and remelts at 197·5–198·5°.

The acid (64 mg.) was refluxed in a stream of CO_2-free N_2 with HI (*d*, 1·7; 3 ml.) and a little red P. CO_2 was evolved as follows. After 0·75 hr., 1·77 mol. equiv.; 1·5 hr., 2·00 mol. equiv.; 2 hr., 2·11 mol. equiv. The decarboxylation product was recovered by extraction with ethyl acetate as a light-brown solid (44 mg.) which was purified by fractional sublimation in a high vacuum at 160–180° giving a colourless solid (26 mg.), m.p. 260–265°, which on crystallization from water gave 3:5-dihydroxy-*p*-toluic acid as colourless needles, m.p. 263·5–265·5° (decomp.) not depressed on admixture with an authentic synthetic specimen, m.p. 267–268° (decomp.) (see p. 626).

Methylation of this product (10 mg.) with ethereal diazomethane, followed by crystallization of the methylated product from benzene, gave colourless woolly needles (7 mg.) of methyl 3:5-dihydroxy-*p*-toluate, m.p. 187·5–188° not depressed on admixture with an authentic synthetic specimen, m.p. 189–190° (see p. 626).

(*b*) *Oxidation of methyl cyclopolate monomethyl ether with hot alkaline* $KMnO_4$. *Isolation of* 4:6-*dimethoxybenzene*-1:2:3:5-*tetracarboxylic acid* (*IV*). Powdered $KMnO_4$ (6·9 g.) was added in portions over a period of 4 hr. to a solution, heated under reflux, of the monomethyl ether of methyl cyclopolate (1·18 g.) in aqueous 0·03 N-KOH (40 ml.). The excess $KMnO_4$ was then destroyed by adding methanol (1 ml.). The MnO_2 was separated by filtration and washed thoroughly with water. The filtrate and washings were acidified to pH 2 with HCl, and $CaCl_2$ (1·5 g.) was added giving a precipitate of calcium oxalate (40 mg.) which was filtered off and discarded. Conc. HCl (3 ml.) was added to the filtrate which was extracted with ethyl acetate (7 × 40 ml.). The washed and dried (Na_2SO_4) extract was evaporated *in vacuo* giving a pale-yellow gummy solid (0·76 g.). This was crystallized from a mixture of ethyl acetate (1 vol.) and benzene (2 vol.) and gave microprisms (0·425 g.), m.p. 221·5–224·5° (decomp.). Two further crystallizations from the same solvents gave very small colourless prisms (0·25 g.), m.p. 225·5–228° (decomp.) with softening from 222°. (Found: loss in weight at 100° *in vacuo*, 5·8. $C_{12}H_{10}O_{10} \rightarrow C_{12}H_8O_9 + H_2O$ requires 5·7% H_2O; on sample dried to constant weight at 110° *in vacuo*: C, 48·5; H, 2·6; OCH_3, 20·8; equiv. by titration 74. $C_{12}H_8O_9$ requires C, 48·7; H, 2·7; $2OCH_3$, 21·0%; equiv., titrating as a tetrabasic acid (di-acid mono-anhydride) 74.) 4:6-*Dimethoxybenzene*-1:2:3:5-*tetracarboxylic acid* is readily soluble in water, ether and ethyl acetate, but is only slightly soluble in benzene. It readily loses 1 mol. water on heating at 100° *in vacuo* or at its melting point with the formation of the anhydride (V).

The acid $C_{12}H_{10}O_{10}$ (96 mg.) was methylated for 2 days with excess ethereal diazomethane. On evaporating the filtered solution to dryness there was obtained a colourless gum (114 mg.) which was very soluble in ether, methanol, benzene and $CHCl_3$ and slightly soluble in light petroleum. It could not be crystallized from any of these solvents or mixtures thereof. It was purified by rapid sublimation in a high vacuum at 180° giving a colourless distillate which set to a waxy solid (80 mg.), m.p. 44–46°. (Found: C, 52·2, 52·2; H, 5·0, 4·8; OCH_3, 49·5. $C_{16}H_{18}O_{10}$ requires C, 51·9; H, 4·9; $6OCH_3$, 50·3%.)

The acid $C_{12}H_{10}O_{10}$ (92·5 mg.) was refluxed in a stream of CO_2-free N_2 with HI (*d*, 1·7; 3 ml.) and a little red P. CO_2 was evolved as follows. After 0·5 hr., 2·04 mol. equiv.; 0·75 hr., 2·61 mol. equiv.; 1·25 hr., 3·03 mol. equiv.; 1·5 hr., 3·13 mol. equiv. The decarboxylation product was recovered by extraction with ethyl acetate (4 × 12 ml.) as a slightly yellow solid (45 mg.) which was purified by sublimation in a high vacuum at 190–200° giving α-resorcylic acid (3:5-dihydroxybenzoic acid) (33 mg.), m.p. 234·5–235·5° (decomp.) not depressed on admixture with an authentic synthetic specimen, m.p. 234–236° (decomp.). The above decarboxylation product (30 mg.), m.p. 234·5–235·5° (decomp.), was methylated in the usual way with dimethyl sulphate (0·2 ml.), K_2CO_3 (0·30 g.) and acetone (7 ml.). The solvent was removed by evaporation and the residue was refluxed for 1 hr. with aqueous 0·1 N-NaOH (6 ml.). The solution was acidified with HCl and extracted with ether. Removal of the solvent gave a solid (25 mg.), m.p. 178–181°, which was purified by crystallization from aqueous methanol and sublimation in a high vacuum at 120°, giving 3:5-dimethoxybenzoic acid as colourless needles (15 mg.), m.p. 182·5–183·5° not depressed on admixture with an authentic synthetic specimen m.p. 182–183°. (Found: C, 59·0; H, 5·3; OCH_3, 33·7. Calc. for $C_9H_{10}O_4$: C, 59·3; H, 5·5; $2OCH_3$. 34·1%.)

(*c*) *Oxidation of cyclopolide monomethyl ether with cold alkaline* $KMnO_4$. *Isolation of* 4-*carboxy*-5:7-*dimethoxy*-6-*methylphthalide*. A suspension of cyclopolide monomethyl ether (0·24 g.) in aqueous N-KOH (5 ml.) and aqueous $KMnO_4$ (2% (w/v); 21 ml. equiv. to 3·9 atoms O) was held at room temperature for 24 hr. with occasional shaking. MnO_2 and excess $KMnO_4$ were removed by addition of Na_2SO_3. The clear liquid was evaporated *in vacuo* to 10 ml., acidified with HCl and extracted with ether (4 × 25 ml.). Removal of the solvent gave colourless gummy needles (195 mg.) which were washed with a little ether leaving a solid residue (71 mg.), m.p. 192–195°. This was recrystallized from water giving 4-*carboxy*-5:7-*dimethoxy*-6-*methylphthalide* as fine colourless needles, m.p. 198–199° not depressed on admixture with an authentic synthetic specimen, m.p. 198–198·5° (see p. 627).

Cyclopaldic acid

Purification. Crude cyclopaldic acid was recrystallized from boiling water (300 ml./g. + charcoal). Yield about 55%, some cyclopolic acid being isolated from the mother liquors. Repeated crystallization from water gave a constant m.p. of 224–225°, but for analysis a pure sample was more readily obtained by sublimation in a high vacuum at 170°.

General properties. Cyclopaldic acid crystallizes from water in long fluffy colourless needles, m.p. 224–225° with sublimation, resetting on cooling and remelting at 222·5–224·5°. It sublimes readily in a high vacuum at 170°. (Found, on a sublimed sample: C, 55·3; H, 4·45; OCH_3, 13·1; *C*-CH_3, 5·4; equiv. by conductometric titration, 118. $C_{11}H_{10}O_6$ requires C, 55·45; H, 4·2; $1OCH_3$, 13·0; $1C$-CH_3, 6·3%; equiv. titrating as a dibasic acid, 119.) It is moderately soluble in ethanol, methanol and ethyl acetate; sparingly soluble in ether, chloroform and hot benzene and very slightly soluble in boiling light petroleum, b.p. 80–100°. It dissolves slowly with slow effervescence in cold aqueous $NaHCO_3$ to give a yellow solution. Its solution in aqueous NaOH is also yellow, the colour deepening on warming, but reverting to its original intensity on recooling. Its aqueous or ethanolic solution gives a deep ruby-red colour with $FeCl_3$, with no suggestion of blue. Brady's reagent quickly gives a gelatinous orange precipitate, which on crystallization from pyridine forms orange needles, m.p. 231–232° (decomp.). Fehling's solution is not reduced even on boiling, though its original blue colour changes to green. Bromine water, when added to a dilute aqueous solution of cyclopaldic acid, gives with the first few drops a buff colour, which on further careful addition is discharged leaving a colourless solution. No precipitate is formed even after adding excess bromine water. When a trace of cyclopaldic acid is exposed in the neck of a test tube containing aqueous NH_3 (*d*, 0·880) it rapidly turns brown and if then dissolved in the aqueous NH_3 it gives a purple colour, fading after 2–3 hr. to orange with a yellow fluorescence (cf. gladiolic acid, Raistrick & Ross, 1952; Grove, 1952). By contrast, if aqueous NH_3 (*d*, 0·880) is added rapidly to solid cyclopaldic acid no colour develops. Its methanolic solution exhibits in ultraviolet light a green fluorescence which is not shown by cyclopolic acid. It gives a pale, quickly fading, yellow colour with bleaching powder. When dissolved in buffer solution, pH 9·2, and treated with a few drops of an aqueous suspension of 2:6-dichloroquinone chloroimide a green colour is formed in 2 min., changing to blue-green after 10 min. and to dark reddish brown after 3 days.

Acetylation of cyclopaldic acid. (*a*) A mixture of cyclopaldic acid (0·20 g.), anhydrous sodium acetate (0·4 g.) and acetic anhydride (1 ml.) was heated for 0·5 hr. in an oil bath held at 150°. Addition of water, after cooling, gave an oil which quickly solidified (0·31 g.). Two crystallizations from aqueous ethanol (50% by vol.) + charcoal gave colourless needles (0·17 g.), m.p. 159°, resetting on cooling and remelting at 158°. (Found: C, 54·0; H, 5·1; OCH_3, 7·1; $COCH_3$, 38·4. $C_{19}H_{20}O_{11}$, i.e. tetra-acetyl derivative of $C_{11}H_{10}O_6$, H_2O, requires C, 53·8; H, 4·75; $1OCH_3$, 7·3; $4COCH_3$, 40·6%.) The *tetra-acetyl derivative* (XXIII) is insoluble in cold aqueous 2N-NaOH and gives no colour with $FeCl_3$ in ethanol or after dilution with water. A yellow precipitate is slowly formed on the addition of Brady's reagent to its ethanolic solution.

(*b*) A mixture of cyclopaldic acid (0·50 g.), anhydrous sodium acetate (0·50 g.) and acetic anhydride (2·5 ml.) was refluxed vigorously for 6·5 hr. in a bath held at 180–190°. The cooled dark-brown solution was treated with water (10 ml.), giving a semi-solid product which was separated and washed well with cold ethanol (8 ml.). The residual brown powder (0·33 g.), m.p. 170–195°, was crystallized from methanol (25 ml.) giving brown crystals (0·24 g.), m.p. 196–198°, which were purified for analysis by further crystallization from methanol (25 ml.), followed by sublimation in a high vacuum at 170–185° and crystallization of the sublimate (0·17 g.) from methanol. The *condensation product* (XXIV*b*) was obtained as almost colourless needles (0·16 g.) m.p. 197·5–198·5°. (Found, on two different samples: C, 58·9, 58·9; H, 4·2, 4·5; OCH_3, 10·5, 10·3. $C_{15}H_{12}O_7$ requires C, 59·2; H, 4·0; $1OCH_3$, 10·2%.) The product dissolves slowly in cold 0·1N-NaOH, more rapidly in 2N-NaOH, or on warming, to give a yellow solution. It gives no colour in ethanolic solution with $FeCl_3$ and no precipitate with Brady's reagent in aqueous ethanol, except on long standing (perhaps as a result of hydrolysis).

The condensation product (0·1293 g.) was hydrolysed by boiling for 5 hr. with N-H_2SO_4 (20 ml.). (Found, by distillation of acetic acid: $COCH_3$, 12·5. $C_{15}H_{12}O_7$ requires $1COCH_3$, 14·1%.) The insoluble colourless solid (0·109 g.), m.p. 268–274°, remaining after the distillation of the acetic acid was collected and purified by fractional sublimation in a high vacuum. The main fraction subliming at 170–200° melted at 275–277° unchanged on crystallization from ethanol. (Found: C, 59·2; H, 3·9; OCH_3, 11·8. $C_{13}H_{10}O_6$ requires C, 59·55; H, 3·8; $1OCH_3$, 11·8%.) The product, *5-formyl-7-methoxy-8-methylcoumarin-6-carboxylic acid* (XXIV*a*) dissolves slowly in $NaHCO_3$ to give a colourless solution, but at once in 2N-Na_2CO_3 or 2N-NaOH to give a yellow solution. It gives no colour with ethanolic $FeCl_3$. Its 2:4-*dinitrophenylhydrazone* was prepared by the addition of Brady's reagent (34 ml.) to a solution of it (21 mg.) in ethanol (21 ml.) giving, after 24 hr., yellow needles (22 mg.), m.p. 230–232° raised to 256–257° with effervescence, after two crystallizations from ethanol. (Found: C, 49·6; H, 3·8; N, 11·9. $C_{19}H_{14}O_9N_4$, H_2O requires C, 49·6; H, 3·5; N, 12·2%.) The compound gives a red Neuberg reaction; it is completely extracted from an ethereal solution by aqueous $NaHCO_3$.

Reaction and degradation products of cyclopaldic acid

(*a*) *Reaction of cyclopaldic acid with hydrazine. Formation of 5-hydroxy-7-methoxy-6-methylphthalazine* (*XXV*)

Cyclopaldic acid (0·24 g.) was treated with hydrazine hydrate (50%; 0·10 ml.). Water (3 ml.) was added and the whole was boiled for 15 min. The supernatant liquid now no longer gave a precipitate with Brady's reagent. The pale-lime coloured rods (0·144 g.), m.p. 260–262°, which separated, were collected and crystallized from ethanol (40 ml.) giving the *condensation product* as very pale-yellow prisms, m.p. 260–262° (decomp.) with preliminary darkening. (Found: C, 63·1; H, 5·45; N, 14·9; OCH_3, 16·3. $C_{10}H_{10}O_2N_2$ requires C, 63·1; H, 5·3; N, 14·7; $1OCH_3$, 16·3%.) *5-Hydroxy-7-methoxy-6-methylphthalazine* is insoluble in cold water or in freshly prepared aqueous 2% $NaHCO_3$, but dissolves slightly in hot water to give a yellow solution. It dissolves in 2N-Na_2CO_3 or in 2N-NaOH to give a nearly colourless

solution. Brady's reagent gives no precipitate in aqueous solution. $FeCl_3$ gives an orange-red colour in ethanolic solution changing to pale brown on addition of water. The *hydrochloride* was prepared by dissolving the pure phthalazine (28 mg.) in warm 2N-HCl (3 ml.), filtering and allowing to cool to room temperature. The long needles (34 mg.), m.p. 248–250° (decomp.), which had a slight pink colour in bulk, were filtered, washed with 2N-HCl and dried in a high vacuum. (Found: C, 52·8; H, 5·0; N, 12·4; Cl, 16·0; OCH_3, 12·9. $C_{10}H_{10}O_2N_2$, HCl requires C, 53·0; H, 4·9; N, 12·4; Cl, 15·7; $1OCH_3$, 13·7%.)

In a repeat experiment, a mixture of cyclopaldic acid (0·121 g.), hydrazine hydrate (50%; 0·05 ml.) and water (1·5 ml.) was boiled for 15 min. CO_2, estimated by absorption in 0·2N-$Ba(OH)_2$, amounting to 66% of 1 mol. CO_2 was evolved, and 5-hydroxy-7-methoxy-6-methylphthalazine (80 mg.), m.p. 258–261° (decomp.), was collected. Recrystallization from ethanol (25 ml.) gave the pure base (47 mg.), m.p. 261–262° (decomp.).

(*b*) *Action of boiling aqueous* 2N-NaOH *on cyclopaldic acid. Isolation of* iso*cyclopaldic acid* (*XVII*; R = R_1 = H)

A solution of cyclopaldic acid (0·112 g.) in aqueous 2N-NaOH (10 ml.) was refluxed for 15 min. The solution was acidified to congo red with 2N-HCl. *iso*Cyclopaldic acid was precipitated as colourless needles (0·065 g.), m.p. 217° (decomp.), which were collected and crystallized from 50% aqueous methanol (7 ml.). The slightly pink needles (53 mg.) were sublimed at 130–140° in a high vacuum to yield a colourless sublimate, m.p. 224–225°, with sublimation and slight effervescence not depressed on admixture with *iso*cyclopaldic acid, m.p. 223·5–224°, prepared from cyclopolic acid by heating with KOH at 150° (see p. 621). A mixture with cyclopaldic acid, m.p. 224–225°, melted at 200–204°. (Found: C, 55·5; H, 4·5. $C_{11}H_{10}O_6$ requires C, 55·5; H, 4·2%.) It dissolved in aqueous $NaHCO_3$ with effervescence to a colourless solution. It gave no yellow precipitate with Brady's reagent. Its aqueous solution gave with $FeCl_3$ a deep reddish-purple colour which is much bluer in tone than that given by cyclopaldic acid and much redder than that given by cyclopolic acid.

(*c*) *Oxidation of cyclopaldic acid with alkaline* H_2O_2

Isolation of oxycyclopaldic acid (6-*formyl*-5-*hydroxy*-3-*methoxy*-4-*methylphthalic acid* (*XXVI*). *Its derivatives and oxidation products.* H_2O_2 ('100 vol.'; 2 ml.) was added to a solution of cyclopaldic acid (0·4 g.) in aqueous 2N-NaOH (40 ml.). After standing at room temperature for 10 min. the solution was added rapidly to 2N-HCl (42 ml.), cooled and extracted at once with ethyl acetate (2 × 80 ml.). The extract was washed with water (5 ml.) and on removal of the solvent there remained a dry, nearly colourless solid (0·38 g.). This was crystallized from ethyl acetate (5 ml.) and benzene (50 ml.) giving colourless needles (0·28 g.), m.p. 136–137°, with slight effervescence. Recrystallization from the same solvents gave pure *oxycyclopaldic acid*, m.p. 138°, with slight effervescence; water is apparently evolved and the melt resets on cooling to remelt at 140–142° without decomposition. (Found: C, 51·8; H, 3·8; OCH_3, 12·3. $C_{11}H_{10}O_7$ requires C, 52·0; H, 4·0; $1OCH_3$, 12·2%.) Oxycyclopaldic acid is soluble in water. The colourless aqueous solution turns yellow on the addition of NaOH, gives a purplish-red colour with $FeCl_3$ and an immediate yellow precipitate with Brady's reagent.

Oxycyclopaldic anhydride. Oxycyclopaldic acid (35 mg.) was heated to 160° in a test tube for 5 min., cooled and sublimed at 100–125° in a high vacuum. The pale-yellow sublimate, m.p. 141–142°, was recrystallized from light petroleum (5 ml., b.p. 80–100°). *Oxycyclopaldic anhydride* (22 mg.) separated as long pale-yellow needles, m.p. 141° with sublimation. (Found: C, 55·9; H, 3·5; OCH_3, 13·2 $C_{11}H_8O_6$ requires C, 55·9; H, 3·4; $1OCH_3$, 13·1%.)

Unlike cyclopaldic acid, cyclopolic acid is not oxidized with alkaline H_2O_2. A solution of cyclopolic acid (0·25 g.) in water (25 ml.) was treated with 2N-NaOH (5 ml.) and H_2O_2 ('100 vol.'; 3 ml.), and was held at room temperature for 3·5 hr. The solution was acidified with HCl and extracted with ether (3 × 20 ml.). On removal of the solvent, unchanged cyclopolic acid (0·20 g.), m.p. 142° (decomp.), remained. It was identified by recrystallization, m.p. 146·5° (decomp.) not depressed on admixture with cyclopolic acid.

Monomethyl oxycyclopaldate methyl ether (*XXVII*) (*methyl*-(2) 6-*formyl*-3:5-*dimethoxy*-4-*methylphthalate*). A mixture of oxycyclopaldic acid (0·10 g.), dimethyl sulphate (1·0 ml.), anhydrous K_2CO_3 (1·0 g.) and dry acetone (20 ml.) was refluxed for 1·5 hr. The mixture, initially colourless, soon turned yellow, then paled gradually until, after 0·5 hr., it became nearly colourless again. Acetone was removed under reduced pressure and the residue was shaken vigorously with water (20 ml.) until all the oil had dissolved. The yellow solution was extracted twice with ethyl acetate to remove neutral impurities, then acidified to congo red and re-extracted twice with ethyl acetate. On removal of the solvent there remained a gummy solid (0·10 g.) which was crystallized twice from water (second time + charcoal) giving *monomethyl oxycyclopaldate methyl ether* (0·035 g.) as colourless rhombic plates which appeared to have a double m.p., 107–109° or 125–127°, and after cooling will remelt at either of these temperatures or sometimes intermediately. (Found: C, 55·4; H, 5·0; OCH_3, 32·8. $C_{13}H_{14}O_7$ requires C, 55·3; H, 5·0; $3OCH_3$, 33·0%.) This mono-ester is soluble in aqueous $NaHCO_3$, gives no colour with NaOH or $FeCl_3$, but gives a yellow crystalline precipitate with Brady's reagent.

Oxidation of monomethyl oxycyclopaldate methyl ether with $KMnO_4$. (i) *Mild oxidation.* A solution of monomethyl oxycyclopaldate methyl ether (0·20 g.) and $NaHCO_3$ (1 g.) in water (20 ml.) was treated with aqueous 0·05M-$KMnO_4$ (20 ml.) and boiled. The purple colour gradually disappeared and further 10 ml. portions of $KMnO_4$ were added until the purple colour remained for 5 min. when a total of 75 ml. had been added. The mixture was cooled, decolorized with $NaHSO_3$, acidified to congo red and extracted with ethyl acetate (2 × 100 ml.). The extracts were washed with a little water (10 ml.) and evaporated. The residual gum (0·23 g.) was crystallized from ethyl acetate (2 ml.) and benzene (25 ml.), adding light petroleum (15 ml., b.p. 60–80°) to the filtrate.

3-*Carbomethoxy*-4:6-*dimethoxy*-5-*methylphthalic acid* (0·142 g.) separated as colourless rosettes of plates, m.p. 135–137°, with gentle effervescence and loss of water; resetting with difficulty on cooling and remelting at 90–100°. Recrystallization did not raise the melting point. (Found: C, 52·4; H, 4·6; OCH_3, 30·7. $C_{13}H_{14}O_8$ requires C, 52·35;

H, 4·7; $3OCH_3$, 31·2%.) This mono-ester does not give a colour with $FeCl_3$ or a precipitate with Brady's reagent. The same product is also obtained when the oxidation is carried out with cold $KMnO_4$ in dilute NaOH solution.

3-Carbomethoxy-4:6-dimethoxy-5-methylphthalic anhydride. The above mono-ester (50 mg.) was heated at 150° for 1 hr. The melt was cooled and crystallized from light petroleum (5 ml., b.p. 60–80°) + charcoal. The *anhydride* (22 mg.) separated as colourless fluffy needles, m.p. 90° or 101–103°, resetting on cooling and remelting at one or other of these temps. (Found: C, 56·1, 56·1; H, 4·5, 4·3; OCH_3, 33·3. $C_{12}H_{12}O_7$ requires C, 55·7; H, 4·3; $3OCH_3$, 33·2%.) The anhydride does not dissolve in aqueous $NaHCO_3$.

Hydrolysis of the mono-ester. The mono-ester (50 mg.) was hydrolysed by boiling with aqueous 2N-NaOH (10 ml.) for 1 hr. The solution was cooled, acidified and extracted with ethyl acetate (2 × 50 ml.). The extracts were washed with water (5 ml.) and evaporated. The residue (30 mg.) was crystallized from ethyl acetate (3 ml.) and benzene (30 ml.). Fine colourless needles (25 mg.) separated, m.p. 198–199°, not depressed on admixture with 4:6-dimethoxy-5-methylbenzene-1:2:3-tricarboxylic acid (VIII), m.p. 198·5–199·5°, obtained by oxidation of methyl cyclopolate monomethyl ether with cold alkaline $KMnO_4$ (see p. 622).

(ii) *Vigorous oxidation.* Monomethyl oxycyclopaldate methyl ether (0·22 g.) was oxidized with hot alkaline $KMnO_4$ under the same conditions as for the oxidation of methyl cyclopolate monomethyl ether with the same reagent (see p. 623, § *b*). The oxidation product obtained (48 mg.), m.p. 224–226·5° (decomp.), did not depress the melting point of the product, 4:6-dimethoxybenzene-1:2:3:5-tetracarboxylic acid, obtained by oxidation of methyl cyclopolate monomethyl ether.

Syntheses

β-Orcinol (2:6-dihydroxy-1:4-dimethylbenzene (XVIII). β-Orcinol was synthesized by the method of Sonn (1931). By crystallization from benzene it was obtained as colourless jagged needles, m.p. 161·5–162·5°. (Sonn records the m.p. as 163°.) (Found: C, 69·2; H, 7·3. Calc. for $C_8H_{10}O_2$: C, 69·5; H, 7·3%.) An aqueous solution of β-orcinol gives a blue colour with a faint red tinge with $FeCl_3$.

β-Orcinol diacetate. Synthetic β-orcinol (30 mg.) was heated at 140° for 0·75 hr. with anhydrous sodium acetate (50 mg.) and acetic anhydride (0·2 ml.). Addition of water precipitated an oil which was extracted with ether. The ethereal extract was washed with 2N-Na_2CO_3 and water. Removal of the solvent from the dried (Na_2SO_4) extract gave a colourless gum (42 mg.) which slowly crystallized on rubbing, m.p. 43·5–44°. It was purified by crystallization from light petroleum, b.p. 40–60°. β-Orcinol diacetate was obtained as thick colourless needles (21 mg.), m.p. 44–44·5° unchanged by recrystallization from ethanol. (Found: C, 64·7; H, 6·4. Calc. for $C_{12}H_{14}O_4$: C, 64·9; H, 6·4%.) The only melting point previously recorded for this substance was 69° by Herzig & Wenzel (1906).

3:5-Dihydroxy-p-toluic acid (3:5-dihydroxy-4-methylbenzoic acid) (X). *p*-Toluic acid was sulphonated according to the method of Asahina & Asano (1933). The Ba salt of the resulting disulphonic acid was isolated as colourless needles. (Found, on three different preparations dried to constant weight at 100° *in vacuo*: Ba, 32·0, 31·1, 30·8. Calc. for $C_8H_6O_8S_2Ba$: Ba, 31·8%.) The Ba salt was converted into the K salt by interaction with K_2CO_3 in aqueous solution. The K salt of the disulphonic acid was converted into 3:5-dihydroxy-*p*-toluic acid by fusion with KOH essentially by the method of Charlesworth & Robinson (1934). It was purified by repeated crystallization from water giving colourless needles, m.p. 267–268° (decomp.). (Asahina & Asano give m.p. 262°, decomp.)

The acid was treated with excess ethereal diazomethane and the methylation product was crystallized from benzene giving methyl 3:5-dihydroxy-*p*-toluate as colourless needles, m.p. 189–190°. (Asahina & Hayashi (1933) give m.p. 190°.) On acetylation of this ester with acetic anhydride and sodium acetate in the usual way and crystallization of the recovered acetate from light petroleum, b.p. 60–80°, *methyl 3:5-diacetoxy-p-toluate* was obtained as colourless needles, m.p. 101–101·5°. (Found: C, 59·1; H, 5·3; OCH_3, 12·0. $C_{13}H_{14}O_6$ requires C, 58·7; H, 5·3; $1OCH_3$, 11·7%.)

3:5-Dihydroxy-4-methylbenzyl alcohol. Excess lithium-aluminium hydride (6·0 g.) was refluxed for 3 hr. with ether (170 ml.) previously dried over Na-K liquid alloy, the solid hydride being crushed occasionally with a glass rod to facilitate solution. The resulting cloudy solution was vigorously stirred and 3:5-dihydroxy-*p*-toluic acid (4·7 g.), m.p. 267–268°, previously dried to constant weight at 100° *in vacuo* and dissolved in a little dry ether, was added dropwise at a rate sufficient to produce a gentle reflux. The thick suspension was stirred and refluxed for a further 3 hr. It was then cooled in an ice bath with constant stirring, and water followed by 2N-H_2SO_4 (250 ml.) was cautiously added. The clear aqueous layer was saturated with NaCl, the ether layer was separated and the aqueous layer was re-extracted with ether (4 × 100 ml.). Removal of the solvent from the combined dried (Na_2SO_4) ether extracts gave light brown slightly resinous needles (3·7 g.), m.p. 145–160°. By cautious washing with a little ether much of the gum was removed and the resulting solid (3·0 g.) melted at 154–156·5°, yield 72%. On crystallization from ethyl acetate-benzene-light petroleum it gave *3:5-dihydroxy-4-methylbenzyl alcohol* as colourless lustrous plates, m.p. 163–163·5°. (Found: C, 62·0; H, 6·4. $C_8H_{10}O_3$ requires C, 62·3; H, 6·5%.) 3:5-Dihydroxy-4-methylbenzyl alcohol is very soluble in water, and its aqueous solution gives a purple-blue colour with $FeCl_3$. With aqueous sodium hypochlorite it gives an intense maroon-red colour which fades only very slowly on standing.

3:5-Diacetoxy-4-methylbenzyl acetate. 3:5-Dihydroxy-4-methylbenzyl alcohol (25 mg.) was acetylated in the usual way at 140° with sodium acetate (40 mg.) and acetic anhydride (0·1 ml.). The crude acetate (43 mg.), m.p. 53·5–55°, recovered on addition of water, on crystallization from light petroleum gave *3:5-diacetoxy-4-methylbenzyl acetate* as thick colourless needles (25 mg.), m.p. 60–60·5°. (Found: C, 59·9; H, 6·1. $C_{14}H_{16}O_6$ requires C, 60·0; H, 5·8%.)

Tri-p-nitrobenzoate of 3:5-dihydroxy-4-methylbenzyl alcohol. A solution of 3:5-dihydroxy-4-methylbenzyl alcohol (15 mg.) in pyridine (0·25 ml.) was refluxed with *p*-nitrobenzoyl chloride (58 mg., 3·2 mol.) at 120° for 20 min. On cooling, colourless needles separated. Water was added and the product was filtered off and washed with water. It was then triturated with 0·5N-NaOH (5 ml.), separated by filtration, washed and dried giving colourless needles (45 mg.), m.p. 254·5–255°. Crystallization from pyridine gave the *tri-p-nitrobenzoate of 3:5-dihydroxy-4-methylbenzyl alcohol* as small colourless needles, m.p. 255·5–256°, resetting and remelting at 254°. (Found: C, 58·1; H, 3·2; N, 7·2. $C_{29}H_{19}O_{12}N_3$ requires C, 57·9; H, 3·2; N, 7·0%.) It is soluble

in acetic acid and pyridine but only slightly soluble in methanol and ethyl acetate.

Di- and mono-carboxylation of 3:5-dihydroxy-4-methylbenzyl alcohol. CO_2 was bubbled through a mixture of 3:5-dihydroxy-4-methylbenzyl alcohol (0·91 g.), glycerol (8·5 ml.) and $KHCO_3$ (6 g.) which was heated under reflux at 150° for 3 hr. After cooling, water (80 ml.) was added and the red-brown solution was saturated with NaCl and extracted with ether (3 × 50 ml.). Removal of the solvent gave neutral, brown, slightly gummy, jagged needles (0·33 g.), m.p. 131–148°, consisting essentially of unchanged 3:5-dihydroxy-4-methylbenzyl alcohol.

4-Carboxy-5:7-dihydroxy-6-methylphthalide. The alkaline ether-extracted aqueous layer was acidified with HCl and re-extracted with ether (3 × 50 ml.). Removal of the solvent gave a dark-brown gummy solid (0·60 g.) which was dissolved in a small volume of methanol and held at 0° for 3 days. A brown solid (80 mg.), m.p. 261·5° (decomp.), separated and was collected. (For treatment of the mother liquors see below.) The solid was purified in the following steps; crystallized from methanol, plates and thick needles (55 mg.), m.p. 265°; sublimed in a high vacuum at 180–190°, small colourless needles (50 mg.), m.p. 276·5–277·5° (decomp.), completely soluble in $NaHCO_3$; crystallized from methanol, plates and a few thick needles, m.p. 265° (decomp.), crystallized from acetone, plates (30 mg.), m.p. 260–261° (decomp.). Further crystallization from acetone effected no change in melting point. When acetone alone was used for the purification of the crude acid a similar initial rise in melting point occurred, followed by a fall to constant melting point of 260–261° with some effervescence and decomposition. (Found, on sample dried at 100° *in vacuo* C, 53·5; H, 3·9. $C_{10}H_8O_6$ requires C, 53·6; H, 3·6%.) This product, *4-carboxy-5:7-dihydroxy-6-methylphthalide*, dissolves with effervescence in $NaHCO_3$ and colourless needles of the sodium salt readily separate from the solution on standing. An ethanolic solution of the free acid gives an intense red-purple colour with $FeCl_3$, redder in tone than that given by salicylic acid.

Methyl ester of 4-carboxy-5:7-dimethoxy-6-methylphthalide (*XVII*; R = R_1 = CH_3). 4-Carboxy-5:7-dihydroxy-6-methylphthalide (35 mg.) was heated under reflux for 1·5 hr. with dimethyl sulphate (0·2 ml.), K_2CO_3 (0·30 g.) and acetone (10 ml.). Evaporation of the solvent and addition of water gave the crude product (27 mg.), m.p. 131–132°, which was purified by sublimation in a high vacuum at 120° giving the *methyl ester of 4-carboxy-5:7-dimethoxy-6-methylphthalide* as colourless fluffy needles (20 mg.), m.p. 139–139·5° resetting on cooling and remelting at 138·5–139°. The melting point was unchanged by crystallization from light petroleum and was not depressed on admixture with the monomethyl ether of methyl *iso*cyclopaldate (see p. 621). (Found: C, 58·6; H, 5·2; OCH_3, 34·7. $C_{12}H_{14}O_6$ requires C, 58·6; H, 5·3; $3OCH_3$, 35·0%.)

4-Carboxy-5:7-dimethoxy-6-methylphthalide (*XVII*; R = CH_3, R_1 = H). The above methyl ester (25 mg.) was refluxed for 0·5 hr. with aqueous 0·5N-NaOH (5 ml.). Acidification with HCl gave colourless needles (21 mg.), m.p. 196–197°. On crystallization from aqueous methanol, *4-carboxy-5:7-dimethoxy-6-methylphthalide* was obtained as colourless silky needles (12 mg.), m.p. 198–198·5° not depressed on admixture with *iso*cyclopaldic acid monomethyl ether (see p. 621). (Found: C, 57·6; H, 5·1; OCH_3, 24·5. $C_{12}H_{12}O_6$ requires C, 57·2; H, 4·8; $2OCH_3$, 24·6%.)

5:7-Dimethoxy-6-methylphthalide (*XIX*). The methanolic mother liquors (see above), from which the bulk of the 4-carboxy-5:7-dihydroxy-6-methylphthalide had been removed, gave on evaporation a little solid and mainly a gum (0·50 g.) which was extracted with cold water (10 ml.). The undissolved brown solid (52 mg.) was separated by filtration and consisted of crude 4-carboxy-5:7-dihydroxy-6-methylphthalide. The aqueous filtrate was acidified with HCl, boiled for 10 min. and the resulting dark-brown deposit (25 mg.) was filtered off and discarded. The acidified filtrate was saturated with NaCl and extracted with ether (3 × 25 ml.). Removal of the solvent gave a soft amber gum (0·35 g.) which was sublimed in a high vacuum at 190–195° giving a slightly gummy sublimate (0·10 g.) still containing 4-carboxy-5:7-dihydroxy-6-methylphthalide which could not be separated by fractional crystallization or fractional sublimation. The gummy sublimate (100 mg.) was therefore methylated with dimethyl sulphate (0·3 ml.), K_2CO_3 (0·3 g.) and acetone (10 ml.). The solvent was removed and the residue was refluxed for 0·5 hr. with 2N-NaOH (10 ml.). Neutral material was removed by extraction with ether and the alkaline liquors were acidified with HCl. The resulting brown precipitate was filtered off and dissolved in ethyl acetate. The ethyl acetate solution was thoroughly extracted with $NaHCO_3$ to remove 4-carboxy-5:7-dimethoxy-6-methylphthalide. Evaporation of the dried ethyl acetate solution gave brown needles (42 mg.) which were purified by sublimation at 125° in a high vacuum giving slightly yellow needles (31 mg.), m.p. 169·5–170°. Crystallization from light petroleum gave *5:7-dimethoxy-6-methylphthalide* as thin colourless lustrous needles (23 mg.), m.p. 172–172·5° not depressed on admixture with the methylated product from the decarboxylation of *iso*cyclopaldic acid with copper chromite and quinoline (see p. 622). (Found: C, 63·6; H, 5·7; OCH_3, 29·5. $C_{11}H_{12}O_4$ requires C, 63·5; H, 5·8; $2OCH_3$, 29·8%.)

5:7-Dimethoxy-4:6-dimethylphthalide (*XIV*). A solution of 5:7-dimethoxy-6-methylphthalide (41 mg.) in glacial acetic acid (0·2 ml.) and chloromethyl methyl ether (0·2 ml.) was heated for 20 hr. at 90–95°. The solvents were removed *in vacuo* over NaOH. The light-brown gummy residue was dissolved in methanol (7 ml.) and dechlorinated by shaking with Adams's catalyst (40 mg.) in an atmosphere of H_2 for 4 hr. The catalyst was removed by filtration. On removal of the solvent there remained a colourless gum which partly crystallized in needles on standing. It consisted of a mixture of the reaction product and unchanged starting material. The mixture was fractionated by alternate fractional sublimation in a high vacuum and crystallization of the sublimate from light petroleum, giving finally *5:7-dimethoxy-4:6-dimethylphthalide* as colourless needles (1·5 mg.), m.p. 98–100° not depressed on admixture with the methylated Clemmensen's reduction product from cyclopolic acid, m.p. 101·5–102° (see p. 621).

SUMMARY

1. Two hitherto undescribed mould metabolic products, *cyclopolic acid* and *cyclopaldic acid*, have been isolated from culture filtrates of each of two different strains of *Penicillium cyclopium* Westling, one of which is a typical green strain, the other a new white variety.

2. Cyclopolic acid, $C_{11}H_{12}O_6$, forms colourless plates, m.p. 147–148° (decomp.). Cyclopaldic acid, $C_{11}H_{10}O_6$, forms colourless fluffy needles, m.p. 224–225°. A number of functional derivatives and degradation products of each acid has been described.

3. Cyclopolic acid is readily oxidized *in vitro* to cyclopaldic acid by heating with potassium periodate in dilute sulphuric acid solution. Both acids have been shown to be hexa-substituted benzene derivatives and to be closely related structurally to two other mould metabolic products, citrinin and mycophenolic acid.

4. Cyclopolic acid is believed to be *5-formyl-4-hydroxy-6-hydroxymethyl-2-methoxy-3-methylbenzoic acid* and cyclopaldic acid is believed to be *5:6-diformyl-4-hydroxy-2-methoxy-4-methylbenzoic acid* or its tautomer.

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