733. *Gladiolic Acid. Part III.* The Structures of Nor*iso*gladiolic Acid, Dihydrogladiolic Acid, and Cyclopolic Acid.*

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The structures of nor*iso*gladiolic acid and dihydrogladiolic acid are established as 5-hydroxy-6-methylphthalide-4-carboxylic acid (II; R = H, R′ = CO_2H) and (in the lactol form) 3-hydroxy-4-hydroxymethyl-7-methoxy-6-methylphthalide (VII*a*; R′ = Me, R″ = H) respectively. *iso*Gladiolic acid is 7-methoxy-6-methylphthalide-4-carboxylic acid (I; R = Me, R′ = CO_2H) (cf. Grove, *Biochem. J.*, 1952, **50**, 648; Brown and Newbold, *J.*, 1953, 1285). Cyclopolic acid is shown on spectroscopic evidence to be (in the lactol form) 3 : 5-dihydroxy-4-hydroxymethyl-7-methoxy-6-methylphthalide (VII*a*; R′ = Me, R″ = OH).

*iso*GLADIOLIC ACID, product of rearrangement of gladiolic acid (VI; R′ = Me, R = R″ = H) by alkali, was formulated by Grove (*Biochem. J.*, 1952, **50**, 648) as (I; R = Me, R′ = CO_2H). This structure was preferred to (II; R = Me, R′ = CO_2H) in which lactonisation had proceeded in the alternative direction because *iso*gladiolic acid was produced by oxidation under acid conditions of 4-formyl-7-methoxy-6-methylphthalide (" deoxygladiolic acid ") (I; R = Me, R′ = CHO) the structure of which was not in doubt. Nevertheless decarboxylation (with simultaneous demethylation) of *iso*gladiolic acid afforded a hydroxy-

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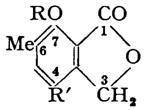
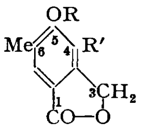
* Part II, *Biochem. J.*, 1953, **54**, 664.

phthalide which gave no colour with ferric chloride and was considered to be (II; $R = R' = H$), indicating that rearrangement had taken place at some point in the reaction sequence (I ; $R = Me$, $R' = CHO$) ⟶ *iso*gladiolic acid ⟶ (II; $R = R' = H$); hence the formula (II; $R = Me$, $R' = CO_2H$) for *iso*gladiolic acid could not entirely be excluded. A similar argument can be applied to the synthesis of *iso*gladiolic acid described by Brown and Newbold (*J.*, 1953, 1285), the final stage of which involves oxidation of (I; $R = Me$, $R' = CH_2{\cdot}OH$) to *iso*gladiolic acid under conditions very similar to those used by Grove (*loc. cit.*) for the oxidation of (I; $R = Me$, $R' = CHO$).

(I)





(II)

Nor*iso*gladiolic acid, obtained on demethylation of *iso*gladiolic acid with hydrobromic acid, was believed by Grove to be (I; $R = H$, $R' = CO_2H$). A systematic study of the infra-red spectra of hydroxyphthalides (Duncanson, Grove, and Zealley, *J.*, 1953, 1331) showed that 7-hydroxyphthalides can be recognised in solution by a characteristic lowering of the phthalide $>C{=}O$ stretching frequency due to intramolecular hydrogen bonding, but that a comparable lowering of frequency observed in the spectra of solidhy droxyphthalides could be caused by intermolecular hydrogen bonding. These conclusions rendered invalid the earlier orientation of nor*iso*gladiolic acid based on results obtained on a Nujol " mull "; examination of the chloroform and dioxan solution spectra of nor*iso*gladiolic acid reveals intramolecular bonding between the hydroxyl and carboxyl substituents (Table 1), the phthalide $>C{=}O$ frequency occurring at the normal unbonded value (1770 $cm.^{-1}$). The characteristic $>C{=}O$ frequency of a 7-hydroxyphthalide is completely absent from the solution spectra which remain unaltered after the lactone ring has been opened by treatment with alkali and the compound recovered by acidification.

Nor*iso*gladiolic acid must therefore be represented by (II; $R = H$, $R' = CO_2H$). Methylation with diazomethane gave some methyl *iso*gladiolate, but the major product was a methyl ether methyl ester (II; $R = Me$, $R' = CO_2Me$) not identical with methyl *iso*gladiolate. An acid-catalysed rearrangement must therefore occur during the demethylation of *iso*gladiolic acid (I; $R = Me$, $R' = CO_2H$) since *iso*gladiolic acid gives methyl *iso*gladiolate (I; $R = Me$, $R' = CO_2Me$) as sole product on methylation with diazomethane. Esterification of nor*iso*gladiolic acid with methanol under Fischer–Speier conditions gave approximately equal amounts of two isomeric methyl esters (II; $R = H$, $R' = CO_2Me$) and (I; $R = H$, $R' = CO_2Me$) oriented by their infra-red spectra (Table 1). Methylation of both esters with diazomethane gave (I; $R = Me$, $R' = CO_2Me$) but the yield, high in the case of (I; $R = H$, $R' = CO_2Me$), was poor with (II; $R = H$, $R' = CO_2Me$). However, with methyl iodide each of the phenol-esters gave exclusively its own methoxy-ester, although yields were low and much starting material was recovered. Decarboxylation of nor*iso*gladiolic acid in quinoline in the presence of copper chromite gave 5-hydroxy-6-methylphthalide (II; $R = R' = H$) identical with the material obtained by decarboxylation of *iso*gladiolic acid, and in low yield 7-hydroxy-6-methylphthalide (I; $R = R' = H$) the structure of which was established by its infra-red spectrum characteristic of a 7-hydroxyphthalide and by methylation to the known 7-methoxy-6-methylphthalide (Brown and Newbold, *loc. cit.*). Methylation of (II; $R = R' = H$) yielded 5-methoxy-6-methylphthalide identical with a synthetic specimen (Gardner and Grove, Part IV, following paper).

It is concluded that while nor*iso*gladiolic acid is best represented as 5-hydroxy-6-methylphthalide-4-carboxylic acid (II; $R = H$, $R' = CO_2H$) in non-hydrolytic solvents, an equilibrium between (II; $R = H$, $R' = CO_2H$) and (I; $R = H$, $R' = CO_2H$) exists in solution under a wide variety of conditions. (I; $R = Me$, $R' = CO_2H$ and CO_2Me) are the stable configurations in the case of the methyl ether and methyl ether methyl ester of nor*iso*gladiolic acid because of steric factors attendant on the presence of the bulky methoxyl substituent; but on demethylation of *iso*gladiolic acid, rearrangement of the resulting

7-hydroxyphthalide (I; R = H, R′ = CO_2H) to the more stable salicylic acid (II; R = H, R′ = CO_2H) takes place. This rearrangement, entailing the opening and closing of a lactone ring in strongly acid solution, may be compared with similar rearrangements in the flavone and *iso*flavone series (Baker, Dunstan, Harborne, Ollis, and Winter, *Chem. and Ind.*, 1953, 277; Whalley, *ibid.*).

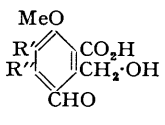
TABLE 1. >C=O *Stretching frequencies of nor*iso*gladiolic acid and derivatives in solution.*

Compound	Solvent	>C=O (cm.$^{-1}$)	Assignment
Nor*iso*gladiolic acid (II; R = H, R′ = CO_2H)	Dioxan	1770 [a]	Phthalide ring
		1686 [a]	Chelated aromatic carboxylic acid
(II; R = H, R′ = CO_2Me)	,,	1769	Phthalide ring
		1680	Chelated aromatic ester
(I; R = H, R′ = CO_2Me)	$CHCl_3$	1746	7-Hydroxyphthalide
		1719	Aromatic ester
(II; R = Me, R′ = CO_2Me)	,,	1766	Phthalide ring
		1735	Hindered aromatic ester
(I; R = Me, R′ = CO_2Me)	,,	1766	Phthalide ring
		1717	Aromatic ester
5-Hydroxy-6-methylphthalide (II; R = R′ = H) ...	Dioxan	1769	Phthalide ring
7-Hydroxy-6-methylphthalide (I; R = R′ = H) ...	$CHCl_3$	1736	7-Hydroxyphthalide
5-Methoxy-6-methylphthalide (II; R = Me, R′ = H)	,,	1757	Phthalide ring
7-Methoxy-6-methylphthalide (I; R = Me, R′ = H)	,,	1761	Phthalide ring

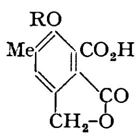
[a] 1763 and 1682 cm.$^{-1}$ respectively in $CHCl_3$.

Dihydrogladiolic acid $C_{11}H_{12}O_5$ was believed to be (III; R′ = Me, R″ = H) since it was converted into 4-formyl-7-methoxy-6-methylphthalide (I; R = Me, R′ = CHO) above its melting point *in vacuo* (Raistrick and Ross, *Biochem. J.*, 1952, **50**, 635), or by boiling dilute mineral acids (Grove, *loc. cit.*); and Clemmensen reduction gave 7-methoxy-4 : 6-dimethylphthalide (I; R = R′ = Me). Further work has shown that (III; R′ = Me, R″ = H) does not account satisfactorily either for the chemistry of dihydrogladiolic acid or for its infra-red spectrum in solution.

Raistrick and Ross showed that acetylation of dihydrogladiolic acid with acetic anhydride–pyridine gave a neutral acetyl derivative (" dihydrogladiolide diacetate ") which they formulated as [I; R = Me, R′ = $CH(OAc)_2$], but all attempts to prepare this derivative from (I; R = Me, R′ = CHO) by an identical acetylation procedure or by a variety of other methods have proved unsuccessful. Secondly, whereas dihydrogladiolic acid is recovered unchanged on acidification after 18 hours in 2N-sodium hydroxide at room temperature or after being heated at 100° in 0·1N-sodium hydroxide, (I; R = Me, R′ = CHO) is also recovered unchanged after similar treatment; the alkaline solutions of (I; R = Me, R′ = CHO) should be equivalent to those of (III; R′ = Me, R″ = H) because back-titration shows the phthalide ring to have been opened under these conditions. Thirdly, by oxidation of dihydrogladiolic acid with alkaline iodine or alkaline hydrogen peroxide, Raistrick and Ross obtained a phthalidecarboxylic acid isomeric with *iso*gladiolic acid; in the present investigation demethylation of this compound gave 6-hydroxy-5-methylphthalide-7-carboxylic acid (IV; R = H) which yields the original oxidation



(III)



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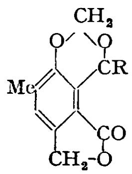

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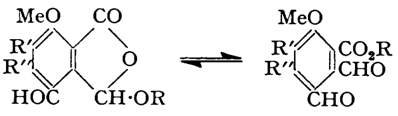
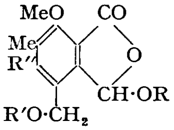
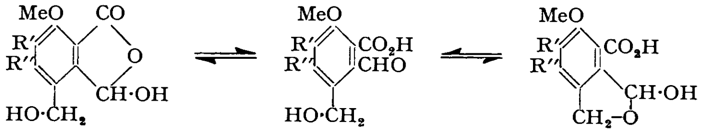


(*a*) (VI) (*b*)



(VIII)



(VII*a*) (VII*b*) (VII*c*)

product on methylation. We have synthesised (IV; $R = H$) by hydrolysis of (V; $R = O$) prepared by the method of Charlesworth, Anderson, and Thompson (*Canad. J. Res.*, 1953, *B*, **31**, 65) in which (V; $R = H_2$) obtained by the action of formaldehyde and hydrochloric acid on 2-hydroxy-*p*-toluic acid was oxidised by chromic oxide. Decarboxylation of (IV; $R = H$) gave the known 6-hydroxy-5-methylphthalide. The dihydrogladiolic acid oxidation product must therefore be 6-methoxy-5-methylphthalide-7-carboxylic acid (IV; $R = Me$) and it is not easy to see how this can arise from (III; $R' = Me$, $R'' = H$), particularly as dihydrogladiolic acid is known not to undergo rearrangement under the mild alkaline conditions employed.

A comprehensive study of the oxidation of (I; $R = Me$, $R' = CHO$) and dihydrogladiolic acid in parallel experiments with a wide variety of conditions and oxidising agents has shown that, while (I; $R = Me$, $R' = CHO$) always gives *iso*gladiolic acid as the initial product, dihydrogladiolic acid gives (IV; $R = Me$) under alkaline, but gladiolic acid (VI; $R' = Me$, $R = R'' = H$) under acid conditions.

These results are best explained on the basis of structure (VII; $R' = Me$, $R'' = H$) for dihydrogladiolic acid. In acid solution where the lactol form (VII*a*; $R' = Me$, $R'' = H$) is the predominant molecular species the hydroxymethyl substituent is the most sensitive to oxidative attack and gladiolic acid is the initial product; in alkaline solution the formyl substituent in the open form (VII*b*; $R' = Me$, $R'' = H$) is attacked first, with the production of (IV; $R = Me$). Strong independent support for structure (VII; $R' = Me$, $R'' = H$) for dihydrogladiolic acid comes from the infra-red spectrum of a dioxan solution (Table 2) which shows $>C=O$ absorption at 1774 cm.$^{-1}$ characteristic of the presence of a phthalide ring and consistent only with (VII*a*; $R' = Me$, $R'' = H$).

Final proof of the structure of dihydrogladiolic acid was obtained as follows. Catalytic reduction of the neutral monoacetyl derivative of gladiolic acid (VI*a*; $R = Ac$, $R' = Me$, $R'' = H$) gave (VIII; $R = Ac$, $R' = R'' = H$) which retained the characteristic lactol acetate infra-red absorption (bands close to 1780 and 1770 cm.$^{-1}$; Table 2). Mild alkaline hydrolysis of this compound under conditions where dihydrogladiolic acid is known to be stable gave dihydrogladiolic acid.

Acetylation of (VIII; $R = Ac$, $R' = R'' = H$) gave a neutral acetyl derivative identical with " dihydrogladiolide diacetate " which must therefore have the structure (VIII; $R = R' = Ac$; $R'' = H$). The infra-red spectrum of this derivative (Table 2) showed a typical ester $>C=O$ absorption band close to 1740 cm.$^{-1}$ consistent with that structure, but incompatible with [I; $R = Me$, $R' = CH(OAc)_2$], since compounds containing the $CH(OAc)_2$ grouping, *e.g.*, 3-acetoxy-4-diacetoxymethyl-7-methoxy-6-methylphthalide, show $>C=O$ absorption close to 1780 cm.$^{-1}$.

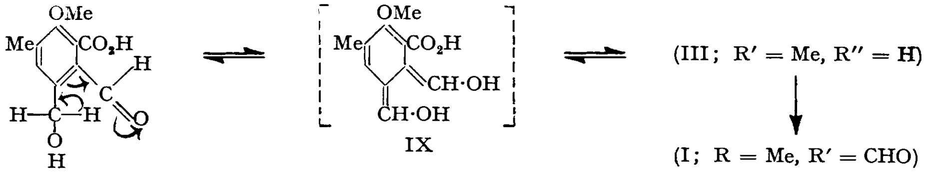
TABLE 2. *Infra-red absorption data for dihydrogladiolic acid and related compounds.*

Compound	$>C=O$ $CHCl_3$	$>C=O$ Solid	-OH Solid	Compound	$>C=O$ $CHCl_3$	$>C=O$ Solid	-OH Solid
(VI*a*; $R = Ac$, $R' = Me$, $R'' = H$)	1786	—	—	" Cyclopolide triacetate " (VIII; $R = R' = Ac$, $R'' = OAc$)	—	1786	—
	—	1768	—		—	1772	—
	1775	—	—		—	1740	—
	1700	1700	—	Dihydrogladiolic acid	1774	1722	3340, 3240
3-Acetoxy-4-diacetoxymethyl-7-methoxy-6-methylphthalide	—	1785	—				
	1776	1765	—	Cyclopolic acid	1768*	1700	—
(VIII; $R = Ac$, $R' = R'' = H$)	—	1776	3490	6-Hydroxy-5-methylphthalide	1756	1734	3280
	—	1764	—	6-Methoxy-5-methylphthalide	1757	—	—
(VIII; $R = R' = Ac$, $R'' = H$)	1786	1796	—				
	1776	1770	—				
	1744	1737	—				

* In dioxan.

[At this point we were informed by Dr. G. T. Newbold (personal communication) that he had obtained similar results and reached similar conclusions in a series of compounds derived from 3-formylopianic acid (VI; $R = H$, $R' = R'' = OMe$) and we agreed to the simultaneous publication of our findings. See Brown and Newbold, *J.*, 1953, 1285.]

The formation of (I; $R = Me$, $R' = CHO$) from dihydrogladiolic acid (see above) involves a novel type of molecular rearrangement resulting in the interchange of the formyl and hydroxymethyl substituents. Although the precise mechanism has not been determined we believe that prototropic rearrangement of (VII*b*; R′ = Me, R″ = H) (present in trace amounts even under acid conditions) can afford the hypothetical intermediate (IX) [its formation in the melt may involve the simultaneous acquisition and release of protons between 2 molecules of (VII*b*)] which under conditions favouring the formation of a stable phthalide ring gives (I; R = Me, R′ = CHO) *via* (III; R′ = Me, R″ = H).


IX

(III; R′ = Me, R″ = H)

(I; R = Me, R′ = CHO)

The formation of 7-methoxy-4 : 6-dimethylphthalide on Clemmensen reduction of dihydrogladiolic acid is explained on the basis of rearrangement to (I; R = Me, R′ = CHO) before the reduction of the formyl group.

Although there is no evidence for a true equilibrium between (VII; R′ = Me, R″ = H) and (III; R′ = Me, R″ = H) in solution at room temperature, as shown by the spectroscopic evidence and the results of the oxidation experiments described above, dihydrogladiolic acid shows keto–lactol tautomerism in aqueous solution and an equilibrium exists between the lactol (VII*a*; R′ = Me, R″ = H) and open-chain (VII*b*; R′ = Me, R″ = H) forms, the pH being the determining factor as in the case of gladiolic acid (Grove, *J.*, 1952, 3345; *idem, Biochem. J.*, 1953, **54** 664).

The ultra-violet absorption (Table 3) in buffer solution of pH 2·9 resembles those of a chloroform solution and of 3-acetoxy-4-diacetoxymethyl-7-methoxy-6-methylphthalide (max. at 297 mμ; log ε 3·73) and indicates the presence of the lactol form (VII*a*; R′ = Me, R″ = H). In alkaline solution the ultra-violet absorption of the open-chain anion is close to that of gladiolic acid (max. at 276 mμ; log ε 3·08) and resembles that of a 2 : 6-disubstituted benzoic acid. The typical absorption of the conjugated benzenoid and carbonyl chromophores is not observed; this may be due to steric factors or to hydration of the formyl substituent either by reaction with the medium or intramolecularly (VII*c*; R′ = Me, R″ = H) (Lüttke, *Chem. Ber.*, 1950, **83**, 571; Hurd and Saunders, *J. Amer. Chem. Soc.*, 1952, **74**, 5324). In ethanol solution the ultra-violet absorption of dihydrogladiolic acid was found to vary with concentration. Thus a $4{\cdot}91 \times 10^{-3}$M-solution showed $\lambda_{\text{max.}}$ 296 mμ (log ε 3·42) and a $2{\cdot}19 \times 10^{-4}$M-solution $\lambda_{\text{max.}}$ 276 mμ (log ε 3·31). This phenomenon is due to dissociation of dihydrogladiolic acid in ethanol, the more dilute solution containing a high proportion of anion.

The formulation of dihydrogladiolic acid as (VII; R′ = Me, R″ = H) suggested that the closely related metabolic product of *Penicillium cyclopium*, cyclopolic acid, might have the structure (VII; R′ = Me, R″ = OH) rather than (III; R′ = Me, R″ = OH) proposed by Birkinshaw, Raistrick, Ross, and Stickings (*Biochem, J.*, 1952, **50**, 610). Strong support for this view comes from the infra-red spectrum of cyclopolic acid in dioxan which shows phthalide ring C=O absorption at 1768 cm.$^{-1}$, consistent only with (VII*a*; R′ = Me, R″ = OH). No bands which can be attributed to the —CO_2H group are observed. Moreover, while cyclopaldic acid (VI; R = H, R″ = OH) gives a green colour in methanol with the titanous chloride reagent of Weygand and Csendes (*Chem. Ber.*, 1952, **85**, 45), similar to that given by salicylaldehyde, cyclopolic acid gives no colour with this reagent. In addition, Birkinshaw *et al.* observed that, while cyclopaldic acid in common with most *o*-hydroxy-aldehydes gave a yellow solution in sodium hydroxide, cyclopolic acid formed a

TABLE 3. *Ultra-violet absorption data for dihydrogladiolic acid.*

Solvent	$\lambda_{\text{max.}}$ (mμ)	log ε
Chloroform	299	3·43
McIlvaine buffer pH 2·9	297	3·31
,, ,, pH 4·0	286—292	3·17
,, ,, pH 5·0	277	3·21
0·1N-Sodium hydroxide	277	3·26

colourless solution. In view of these observations, which favour (VII; R′ = Me, R″ = OH) the intense purple ferric reaction of cyclopolic acid which is replaced by an orange colour in the thiosemicarbazone, the conductometric titration of cyclopolic acid as a dibasic acid and the resistance shown by cyclopolic acid towards oxidation by alkaline hydrogen peroxide are surprising and might be taken to indicate an equilibrium with (III; R′ = Me, R″ = OH) in aqueous solution at room temperature under the influence of certain reagents. Nevertheless we consider the infra-red evidence to be conclusive in showing that cyclopolic acid has the structure (VII; R′ = Me, R″ = OH) in non-hydrolytic solvents. On this structure the production of 4-hydroxy-7-iodo-6-methoxy-5-methylphthalide by the action of alkaline iodine on cyclopolic acid (Ross, Thesis, London, 1951) is readily understood and it is no longer necessary to postulate the intermediate formation and subsequent rearrangement of 3-iodo-4-methoxy-5-methylphthalaldehyde in this reaction. The formation of cyclopolide, 4-formyl-5-hydroxy-7-methoxy-6-methylphthalide, when cyclopolic acid is boiled with dilute mineral acids, and of 5 : 7-dimethoxy-4 : 6-dimethylphthalide on Clemmensen reduction of the acid followed by methylation, has then to be explained on the basis of a molecular rearrangement in acid solution similar to that postulated for dihydrogladiolic acid. The triacetyl derivative of cyclopolic acid, formulated by Birkinshaw *et al.* as a derivative of cyclopolide, resembles (VIII; R = R′ = Ac, R″ = H) in showing ester >C=O absorption at 1740 cm.$^{-1}$ (Table 2) and is therefore more correctly represented by (VIII; R = R′ = Ac, R″ = OAc).

EXPERIMENTAL

M. p.s are corrected. Microanalyses are by Messrs. W. Brown and A. G. Olney. Infra-red spectra were measured with a Grubb–Parsons S 3A spectrometer through which dry air was continuously circulated. Ultra-violet spectra were measured using a Unicam S.P. 500 spectrophotometer and 1-cm. path-length, except for ethanol solutions of dihydrogladiolic acid where the Hilger medium quartz spectrograph was used (path-length 0·1—1 cm.). B.D.H. "Spectroscopic ethanol" was used in these experiments. Comparison of the infra-red spectra was used to check identifications by mixed m. p. determination in all cases of suspected identity.

Pure specimens of cyclopolic acid, m. p. 147—148° (decomp.), and cyclopaldic acid, m. p. 224—225°, were kindly provided by Prof. H. Raistrick, F.R.S. Acetylation of cyclopolic acid with acetic anhydride–pyridine gave the triacetyl derivative ("cyclopolide triacetate") (VIII; R = R′ = Ac, R″ = OAc), m. p. 114°.

Dihydogladiolic acid (VII; R′ = Me, R″ = H) was obtained only in very low and variable yield by extraction of cultures of *Penicillium gladioli* McCull and Thom (N.C.T.C., 3994) and formed colourless prisms, m. p. 135—136° (decomp.), from ethyl acetate–benzene (Raistrick and Ross, *loc. cit.*) (Found: C, 59·0; H, 5·3. Calc. for $C_{11}H_{12}O_5$: C, 58·9; H, 5·4%).

After 18 hr. in 2N-sodium hydroxide at room temperature, or after 45 min. at 100° with 0·1N-sodium hydroxide, dihydrogladiolic acid was precipitated unchanged on acidification of the cooled solutions with hydrochloric acid. Dihydrogladiolic acid underwent degradation with the formation of both neutral and acidic material under reflux (1 hr.) with 2N-sodium hydroxide. Acetylation of dihydrogladiolic acid with acetic anhydride–pyridine according to Raistrick and Ross (*loc. cit.*) gave colourless needles, m. p. 70°, of "dihydrogladiolide diacetate" identical with (VIII; R = R′ = Ac, R″ = H).

Dihydrogladiolic acid (3·0 mg.) dissolved in 2N-hydrochloric acid (0·3 ml.) at 100°. After a few minutes colourless needles began to separate from the slightly yellow solution and after 15 min. these were filtered off (2·5 mg.) and identified as (I; R = Me, R′ = CHO).

4-Formyl-7-methoxy-6-methylphthalide (I; R = Me, R′ = CHO), m. p. 173°, was prepared by reduction of gladiolic acid (Grove, *loc. cit.*). Careful acidification of the alkaline solutions obtained by heating (I; R = Me, R′ = CHO) with excess of 0·1N-sodium hydroxide at 100° for 45 min. precipitated (I; R = Me, R′ = CHO) unchanged. This treatment with alkali has previously been shown to open the phthalide ring of (I; R = Me, R′ = CHO). It was also recovered unchanged after 2 days at room temperature in acetic anhydride–pyridine, treatment with acetic anhydride and concentrated sulphuric acid (1 drop), and heating at 100° for 4 hr. with acetic anhydride and acetic acid (cf. acetylation of gladiolic acid; Grove, *loc. cit.*). Clemmensen reduction of (I; R = Me, R′ = CHO) as described for gladiolic acid gave needles, m. p. 112°, identical with 7-methoxy-4 : 6-dimethylphthalide.

3-*Acetoxy-4-hydroxymethyl-7-methoxy-6-methylphthalide* (VIII; R = Ac, R′ = R″ = H).—3-Acetoxy-4-formyl-7-methoxy-6-methylphthalide (80 mg.), in ethanol (10 ml.), was added to a reduced platinum oxide catalyst (15 mg.), in ethanol (5 ml.), and the hydrogenation carried out at 16°. Rapid uptake of 1 mol. (10 min.) was followed by a slower uptake of a second mol. (1 hr.). Reduction was stopped, the catalyst filtered off, and the solvent removed. Crystallisation of the crude product from ethanol (0·7 ml.) furnished unchanged starting material (23 mg.; m. p. 139°). Dilution of the filtrate to 2 ml. with water yielded a further small fraction of impure starting material (2 mg.; m. p. 130—136°), and the aqueous-ethanolic filtrate was then made alkaline with sodium hydrogen carbonate and extracted with ether. Acidification of the aqueous layer and recovery yielded a gummy acid (10 mg.). The neutral ethereal extract furnished a gum which after two crystallisations from benzene–light petroleum (b. p. 60—80°) gave 3-*acetoxy-4-hydroxymethyl-7-methoxy-6-methylphthalide*, needles (16 mg.), m. p. 85° (Found: C, 58·55; H, 5·5. $C_{13}H_{14}O_6$ requires C, 58·6; H, 5·3%). Acetic anhydride–pyridine at 20° gave the *acetyl* derivative (VIII; R = R′ = Ac, R″ = H), colourless needles (from methanol), m. p. 70° (Found: C, 58·5; H, 5·4. $C_{15}H_{16}O_7$ requires C, 58·4; H, 5·2%).

Hydrolysis of (VIII; R = Ac, R′ = R″ = H).—The compound (3·1 mg.) dissolved when kept in 2N-sodium hydroxide (0·1 ml.). After 4 hr. at room temperature acidification with concentrated hydrochloric acid precipitated dihydrogladiolic acid as needles (2·2 mg.), m. p. 132°.

Oxidation Experiments with (a) *Dihydrogladiolic Acid and* (b) 4-*Formyl-7-methoxy-6-methylphthalide* (I; R = Me, R′ = CHO).—(A) *Alkaline hydrogen peroxide.* (*a*) Dihydrogladiolic acid yielded 6-methoxy-5-methylphthalide-7-carboxylic acid (IV; R = Me), m. p. 216°, identical with the phthalidecarboxylic acid obtained by Raistrick and Ross (*loc. cit.*). When (IV; R = Me) was heated at 100° for 20 min. with hydrobromic acid (*d* 1·25) colourless needles of 6-*hydroxy-5-methylphthalide-7-carboxylic acid*, m. p. 198°, identical with a synthetic specimen, separated (Found: C, 57·7; H, 4·0. $C_{10}H_8O_5$ requires C, 57·7; H, 3·9%).

(*b*) The phthalide (I; R = Me, R′ = CHO) (27·4 mg.) was dissolved in 0·108N-sodium hydroxide (4·05 ml.) at 100° (45 min.) and then hydrogen peroxide (20-vol.; 2 ml.) was added to the cooled solution. After 24 hr. an additional 1 ml. of hydrogen peroxide was added and the mixture set aside for a further 24 hr. Back-titration with 0·115N-hydrochloric acid used up 1·41 ml., whence the acidity liberated during solution and oxidation $\equiv$ 2·53 ml. of 0·108N-sodium hydroxide (Calc. for 2 equiv., 2·46 ml.). The solution was made strongly acid and after 4 hr. at 0° the precipitated solid was dissolved in ether and the ethereal solution extracted with sodium hydrogen carbonate solution. Acidification of the carbonate extract, ether-extraction, and recovery yielded 7-methoxy-6-methylphthalide-4-carboxylic acid (I; R = Me, R′ = CO_2H) (m. p. 200—230°), crystallising from water in needles (10·6 mg.), m. p. 234°.

(B) *Alkaline iodine.* (*a*) Dihydrogladiolic acid (42·8 mg.) yielded (IV; R = Me) (12·2 mg.) (Raistrick and Ross, *loc. cit.*).

(*b*) The phthalide (I; R = Me, R′ = CHO) (27·0 mg.) in warm methanol (3 ml.) was treated with 0·108N-sodium hydroxide (15·0 ml.) and 0·1N-iodine (10·0 ml.) and kept for 18 hr. at room temperature. Back-titration with 0·1N-sodium thiosulphate after addition of excess of hydrochloric acid showed the iodine consumed to be equivalent to 1·01 atoms of oxygen. Extraction of the acid solution with ethyl acetate and recovery furnished (I; R = Me, R′ = CO_2H) (10·0 mg.), m. p. 236°.

(C) *Chromic oxide.* (*a*) Dihydrogladiolic acid (17 mg.) in acetic acid (2 ml.) was treated with chromic oxide (12 mg.) in water (0·5 ml.). After 12 hr. the clear solution was diluted with water and extracted with chloroform, furnishing a gum which was sublimed at 160°/10^{-2} mm. Crystallisation of the sublimate from water gave gladiolic acid, m. p. 158—160° (4·5 mg.).

(*b*) On oxidation of (I; R = Me, R′ = CHO) (20 mg.) under identical conditions, needles (5 mg.) of (I; R = Me, R′ = CO_2H), m. p. 234°, had crystallised from the mixture after 12 hr. On dilution with water the filtrate deposited a further 5 mg. of (I; R = Me, R′ = CO_2H).

(D) *Acid potassium permanganate.* (*a*) Dihydrogladiolic acid (20 mg.) in 2N-sulphuric acid (3 ml.) at 90° was oxidised by rapid addition of potassium permanganate (15 mg.) in water. The solid product (5 mg.), m. p. 140—150°, which separated on cooling gave the green colour with ammonia solution (*d* 0·880) characteristic of gladiolic acid, but resisted further purification. Extraction of the filtrate with ether and recovery furnished a semi-solid mass (11 mg.) from which needles of gladiolic acid (6 mg.), m. p. 154°, were obtained by crystallisation from water.

(*b*) The phthalide (I; R = Me, R′ = CHO) furnished (I; R = Me, R′ = CO_2H) as described by Grove (*loc. cit.*).

(E) *Acid hydrogen peroxide.* (*a*) Dihydrogladiolic acid (10 mg.) was kept in glacial acetic acid (1 ml.) and hydrogen peroxide (20-vol; 1·5 ml.) for 24 hr. at room temperature. Dilution with water and extraction with chloroform afforded a gum which gave a colourless sublimate at 160—180°/10^{-2} mm. Separation of the sublimate into neutral and acidic fractions by dissolution in ether and extraction with sodium hydrogen carbonate furnished (I; R = Me, R′ = CHO) (1·5 mg.) and gladiolic acid (1·1 mg.).

6-*Hydroxy*-5-*methylphthalide*.—6-Methoxy-5-methylphthalide (120 mg.) (Charlesworth, Rennie, Sinder, and Yan, *Canad. J. Res.*, 1945, *B*, **23**, 17) was heated under reflux for 5 min. with hydrobromic acid (3 ml.; *d* 1·25), and the solution was diluted with water (10 ml.) and extracted with ether. The ethereal solution was extracted with 2N-sodium hydroxide; on acidification with concentrated hydrochloric acid a little amorphous material separated and was filtered off and discarded, and the clear filtrate extracted with ether. The crude product (75 mg.; m. p. 190—200°) crystallised from ethyl acetate in needles, m. p. 210° (Found: C, 65·9; H, 4·9. Calc. for $C_9H_8O_3$: C, 65·85; H, 4·9%). Charlesworth *et al.* (*loc. cit.*, 1953) give m. p. 205°.

6-*Hydroxy*-5-*methylphthalide*-7-*carboxylic Acid* (IV; R = H).—The lactone (V; R = O) of 6-hydroxymethyl-8-methyl-4-oxobenzo-1 : 3-dioxan-5-carboxylic acid (Charlesworth, Anderson, and Thompson, *loc. cit.*) was hydrolysed quantitatively by 3N-sodium hydroxide under reflux in 1·5 hr. Acidification and crystallisation from water gave 6-*hydroxy*-5-*methylphthalide*-7-*carboxylic acid*, needles, m. p. 198° [Found: C, 57·7; H, 3·9%; *M* (Rast), 238. $C_{10}H_8O_5$ requires C, 57·7; H, 3·9%; *M*, 208]. Methylation with methyl sulphate in 10% sodium hydroxide, followed by warming for 1 hr. on a steam-bath with sodium hydroxide to hydrolyse any methyl ester formed, gave 6-methoxy-5-methylphthalide-7-carboxylic acid (IV; R = Me), m. p. 214°, identical with the product of oxidation of dihydrogladiolic acid by alkaline hydrogen peroxide.

The acid (IV; R = H) was decarboxylated by quinoline with a copper chromite catalyst at 175—190° (2½ hr.) as described for (II; R = H, R′ = CO_2H) (see below) and the product worked up similarly. Carbon dioxide corresponding to 1·08 mols. was collected. Sublimation of the product and crystallisation from water gave 6-hydroxy-5-methylphthalide.

5-*Hydroxy*-6-*methylphthalide*-4-*carboxylic Acid* (*Nor*iso*gladiolic Acid*) (II; R = H, R′ = CO_2H).—The specimen prepared previously (Grove, *loc. cit.*) melted at 254—256° (decomp.). Specimens prepared later by the same method have melted at 276° (decomp.) under the same conditions, but no significant differences in analysis or infra-red spectra (in Nujol "mull" or in dioxan solution) have been detected. Grinding the higher-melting material lowered the m. p. to 268° (decomp.), and it appears that the m. p. depends on the particle size. The yield, m. p., and infra-red spectrum of the product were unaltered when the time of heating *iso*gladiolic acid with hydrobromic acid was reduced to 10 min. The acid (II; R = H, R′ = CO_2H) (49 mg.) was heated on the steam-bath for 1 hr. with an excess of 0·1N-sodium hydroxide. Back-titration with 0·1N-hydrochloric acid gave equiv. 104 [Calc. for $C_{10}H_8O_5$: equiv., 104 (dibasic acid)]. Acidification to Congo-red precipitated colourless needles (47 mg.), m. p. 268° (decomp.) raised to 274° (decomp.) by crystallisation from ethanol. The infra-red spectrum was identical with that of the starting material and the mixed m. p. showed no depression.

Methyl 7-*Methoxy*-6-*methylphthalide*-4-*carboxylate* (*Methyl* iso*Gladiolate*) (I; R = Me, R′ = CO_2Me).—*iso*Gladiolic acid and ethereal diazomethane in the presence of methanol gave a homogeneous colourless solid, m. p. 136—138°. Recrystallisation from methanol gave needles, m. p. 142—143°, identical with methyl *iso*gladiolate prepared under Fischer–Speier conditions (Grove, *loc. cit.*). The ester showed dimorphism. The two forms had the same m. p. and appearance, and the same infra-red spectra in chloroform solution, but different infra-red spectra in the solid state. Form A, usually obtained by the Fischer–Speier method from (I; R = Me, R′ = CO_2H) showed bands at 1773 s, 1705 s, 1602 m, 1499 m, 1330 s, 1313 m, 1283 m, 1247 s, 1217 s, 1196 w, 1175 m, 1159 w, 1107 s, 1028 m, 1002 s, 967 m, 935 w, 909 m, 880 w, 797 m, 780 m, 767 w, 729 w. Form B, generally obtained by methylation of (II; R = H, R′ = CO_2H) showed bands at 1771 s, 1736 s, 1618 m, 1593 m, 1496 m, 1310 s, 1275 m, 1241 m, 1208 s, 1188 m, 1111 m, 1101 m, 1086 s, 1022 s, 1001 s, 958 m, 939 w, 913 w, 880 w, 796 m, 775 w, 769 w, 730 w. Forms A and B were interconvertible by seeding of methanol solutions with the appropriate crystalline modification.

Decarboxylation of (II; R = H, R′ = CO_2H).—The acid (0·79 g.) was decarboxylated in quinoline at 180° in the presence of copper chromite, and the products were worked up as described by Grove (*loc. cit.*). Ether-extraction of the acidified filtrate yielded a brown solid (*ca.* 0·4 g.). Sublimation at room temperature and 10^{-2} mm. gave an oil which did not solidify. At 80—100°/10^{-4} mm. an orange solid (28 mg.) was obtained which on recrystallisation from methanol formed colourless needles of 7-*hydroxy*-6-*methylphthalide* (I; R = R′ = H), m. p. 127° (Found: C, 65·7; H, 5·1. $C_9H_8O_3$ requires C, 65·85; H, 4·9%). It gave an intense blue colour with ferric chloride in ethanol. With ethereal diazomethane in the presence of methanol it gave needles, m. p. 118°, identical with 7-methoxy-6-methylphthalide (I; R = Me, R′ = H) kindly supplied by Dr. G. T. Newbold.

The unsublimed material (321 mg.) crystallised from ethanol as brown needles, m. p. 235°, purified by sublimation at 160—180°/10^{-5} mm. followed by recrystallisation from ethanol, giving colourless needles, m. p. 244°, of 5-hydroxy-6-methylphthalide (II; R = R′ = H) identical with a specimen obtained by decarboxylation (and simultaneous demethylation) of *iso*gladiolic acid. The infra-red spectrum was distinct from that of 6-hydroxy-5-methylphthalide. With ethereal diazomethane (II; R = R′ = H) gave colourless needles (from water), m. p. 144°, identical with a synthetic specimen of 5-methoxy-6-methylphthalide (Gardner and Grove, Part IV, following paper).

Methylation of (II; R = H, R′ = CO_2H).—(a) *With diazomethane.* The acid (100 mg.) in methanol was kept with excess of ethereal diazomethane for 2 days at room temperature. Fractional crystallisation of the crude product from methanol yielded (I; R = Me, R′ = CO_2Me) (30 mg.), m. p. 142°, and *methyl* 5-*methoxy*-6-*methylphthalide*-4-*carboxylate* (II; R = Me, R′ = CO_2Me), colourless needles, m. p. 132° (53 mg.) (Found: C, 61·0; H, 5·4. $C_{12}H_{12}O_5$ requires C, 61·0; H, 5·1%). The infra-red spectrum of this compound both in the solid state and in solution differed from (I; R = Me, R′ = CO_2Me) (both forms A and B), and a mixed m. p. determination with (I; R = Me, R′ = CO_2Me) showed a 15° depression.

(b) *With methanolic hydrogen chloride.* The acid (75 mg.), heated under reflux for 2 hr. with methanol (30 ml.) containing 10% of hydrogen chloride, yielded a mixture, separated by chromatography of its benzene solution on a short column of neutral alumina activated at 180°. (i) Elution with benzene and crystallisation from methanol gave colourless needles (36 mg.) of *methyl* 5-*hydroxy*-6-*methylphthalide*-4-*carboxylate* (II; R = H, R′ = CO_2Me), m. p. 212° (Found: C, 59·1; H, 4·75. $C_{11}H_{10}O_5$ requires C, 59·45; H, 4·5%). It gave a reddish-purple colour in ethanol with ferric chloride. The ester (20 mg.) in methanol (4 ml.), treated for 1 day at 20° with excess of ethereal diazomethane, gave (I; R = Me, R′ = CO_2Me) (9 mg.), m. p. 142° after three crystallisations from methanol. The mother-liquors contained a solid (8 mg.), m. p. 120—138°, which resisted purification by fractional crystallisation. The ester (II; R = H, R′ = CO_2Me) (28 mg.), acetone (5 ml.), potassium carbonate (0·2 g.) and methyl iodide (0·2 ml.) were heated under reflux for 3 hr. Water (5 ml.) was added and the solution extracted with ether. Removal of the solvent and recrystallisation of the residue (8 mg.) gave (II; R = Me, R′ = CO_2Me), m. p. 128°. Acidification of the aqueous layer precipitated starting material (14 mg.). No improvement in yield was obtained by using a larger excess of methyl iodide and heating for 8·5 hr.

(ii) Elution of the more strongly adsorbed material with benzene and 1% of methanol, followed by crystallisation from benzene or methanol, gave colourless prisms (24 mg.) of *methyl* 7-*hydroxy*-6-*methylphthalide*-4-*carboxylate* (I; R = H, R′ = CO_2Me), m. p. 197° (Found: C, 59·8; H, 4·8. $C_{11}H_{10}O_5$ requires C, 59·45; H, 4·5%). It gave an intense purple colour in ethanol with ferric chloride. The ester (20 mg.) in methanol (5 ml.), treated for 2 days at 20° with excess of ethereal diazomethane, gave (I; R = Me, R′ = CO_2Me) (16 mg.), m. p. 142°, on concentration of the solvent. The ester (I; R = H, R′ = CO_2Me) (25 mg.), potassium carbonate (0·2 g.), acetone (5 ml.), and methyl iodide (0·2 ml.) were heated under reflux for 8·5 hr. The acetone was removed *in vacuo*, water (8 ml.) was added, and the mixture shaken for 10 min. The residual solid (5 mg.) was collected and recrystallised from methanol, to give (I; R = Me, R′ = CO_2Me), m. p. 144°. Acidification of the filtrate, extraction with ether, and recovery gave starting material (13 mg.)

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