760. *Griseofulvin. Part II.* Oxidative Degradation.*

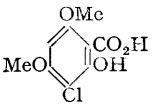
By John Frederick Grove, Doreen Ismay, J. MacMillan, T. P. C. Mulholland, and M. A. Thorold Rogers.

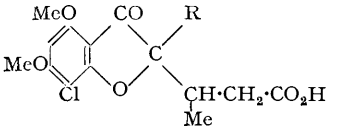
The observation by Oxford *et al.* (*Biochem. J.*, 1939, **33**, 240) that permanganate oxidation of griseofulvin and griseofulvic acid gave 3-chloro-2-hydroxy-4 : 6-dimethoxybenzoic acid and 7-chloro-2-hydroxy-4 : 6-dimethoxycoumaranone-2-β-butyric acid (II; R = OH) has been confirmed and the former compound obtained on further permanganate oxidation of the latter. Oxidation of griseofulvic acid with alkaline hydrogen peroxide gives 7-chloro-4 : 6-dimethoxycoumaranone-2-β-butyric acid (II; R = H) from which (II; R = OH) is obtained on permanganate oxidation. Alkaline hydrolysis of (II; R = H) yields a dibasic acid, $C_{13}H_{13}O_6Cl$, and this same compound is also produced by oxidation of norgriseofulvic acid with alkaline hydrogen peroxide. Oxidation of griseofulvic acid with yellow mercuric oxide or of decarboxygriseofulvic acid with air under alkaline conditions results in the formation of a chlorohydroxydimethoxymethyldibenzofuran. 3-Methoxy-2 : 5-toluquinone is formed when griseofulvin but not *iso*griseofulvin or griseofulvic acid is oxidised with chromic oxide in acetic acid. The significance of these oxidation products in the general problem of the structure of griseofulvin is discussed.

By oxidation of griseofulvin in acetone with a large excess of potassium permanganate, Oxford, Raistrick, and Simonart (*Biochem. J.*, 1939, **33**, 240) obtained two monobasic acids which they considered to be 3-chloro-2-hydroxy-4 : 6-dimethoxybenzoic (I) and 7-chloro-2-hydroxy-4 : 6-dimethoxycoumaran-3-one-2-β-butyric acid (II; R = OH). Both acids were stated to be formed from griseofulvic acid under the same conditions, but decarboxygriseofulvic acid gave only the former. The structures of both these oxidation products

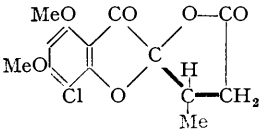
* Part I, preceding paper.

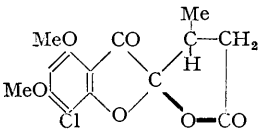
are considered to be correct for reasons which are discussed in Part III (following paper). It is sufficient to say here that we find, in agreement with Oxford *et al.*, that 7-chloro-2-hydroxy-4 : 6-dimethoxycoumaranone-2-β-butyric acid readily undergoes lactonisation. Two isomeric lactones, $C_{14}H_{13}O_6Cl$, however, have been isolated. Lactone A, m. p. 220°, obtained by sublimation of (II; R = OH) *in vacuo* and presumably identical with the lactone, $C_{14}H_{13}O_6Cl$, m. p. 220°, described by Oxford *et al.* which they obtained by the action of acetic anhydride in pyridine on (II; R = OH), is apparently optically inactive. Using acetic anhydride–pyridine, we failed to isolate lactone A but obtained in its place lactone B, $C_{14}H_{13}O_6Cl$, m. p. 178—180°, $[\alpha]_D^{20}$ — 15·5°. Lactone B also results from the action of concentrated sulphuric acid on (II; R = OH). The two lactones are considered to be the diastereoisomers (III) and (IV); racemisation at $C_{(2)}$ of (II; R = OH) could occur *via* the open diketo-form during preparation or lactonisation.

(I) 



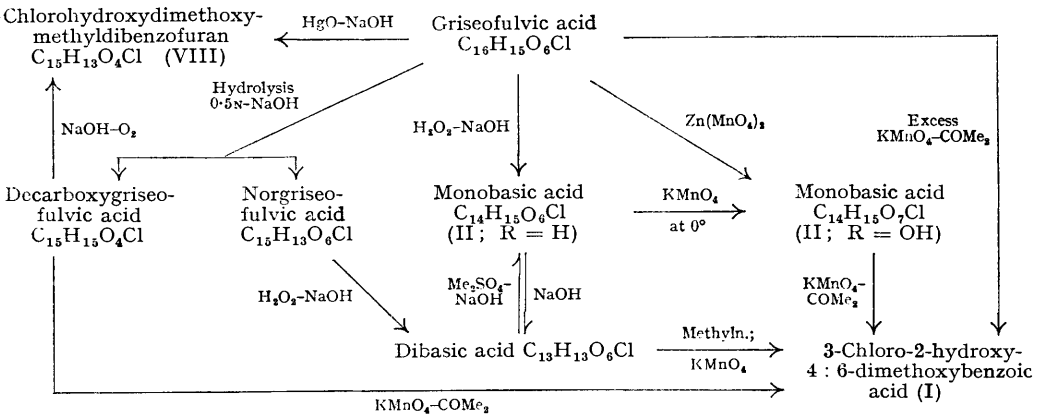
(II)

(III) 



(IV)

7-Chloro-2-hydroxy-4 : 6-dimethoxycoumaranone-2-β-butyric acid (II; R = OH), which gives 3-chloro-2-hydroxy-4 : 6-dimethoxybenzoic acid (I) on further oxidation with permanganate, has been made in a variety of ways including the oxidation of griseofulvic acid with cold alkaline permanganate, hot chromic acid, or cold alkaline hypobromite. The lactone A sometimes accompanies or may replace the acid according to methods used in working up of the crude oxidation products. Only very small yields of 7-chloro-2-hydroxy-4 : 6-dimethoxycoumaranone-2-β-butyric acid are obtained when the permanganate oxidation of griseofulvin is conducted as described by Oxford *et al.*, but the yield is very much improved (approx. 30%) by use of smaller quantities of zinc permanganate in neutral solution. Volatile acids are not produced in significant amounts during any of the oxidations with permanganate described above, nor are saturated dibasic acids.



A variety of new conditions for the oxidation of griseofulvin and its derivatives have been studied (see Scheme). The action of alkaline hydrogen peroxide on griseofulvic acid yielded a monobasic acid, $C_{14}H_{15}O_6Cl$, which closely resembles (II; R = OH) in its ultraviolet absorption spectrum (Fig. 1), but does not appear to have an alcoholic hydroxyl group, as judged by its infra-red spectrum. This acid is rapidly oxidised by ice-cold

alkaline permanganate to (II; R = OH) and is therefore considered to be 7-chloro-4 : 6-dimethoxycoumaranone-2-β-butyric acid (II; R = H); it is in fact an intermediate in the formation of the acid (II; R = OH) by permanganate oxidation of griseofulvin and is present in small quantities in the products. This has not been shown directly owing to difficulty in the separation of the two acids by fractional crystallisation but alkaline hydrolysis of the crude oxidation product (see Part III, following paper) gives, in very low yield, the dibasic acid $C_{13}H_{13}O_6Cl$ (see below) which results from alkaline hydrolysis of (II; R = H).

Oxidation of the dibasic norgriseofulvic acid, $C_{15}H_{13}O_6Cl$, both by hydrogen peroxide and by ice-cold alkaline permanganate gives a new dibasic acid, $C_{13}H_{13}O_6Cl$, containing one methoxyl group. This acid can be made by hydrolysis of 7-chloro-4 : 6-dimethoxycoumaranone-2-β-butyric acid by alkali in an atmosphere of nitrogen. The fragment C_2 has been lost from norgriseofulvic acid and this is the same loss as takes place in the

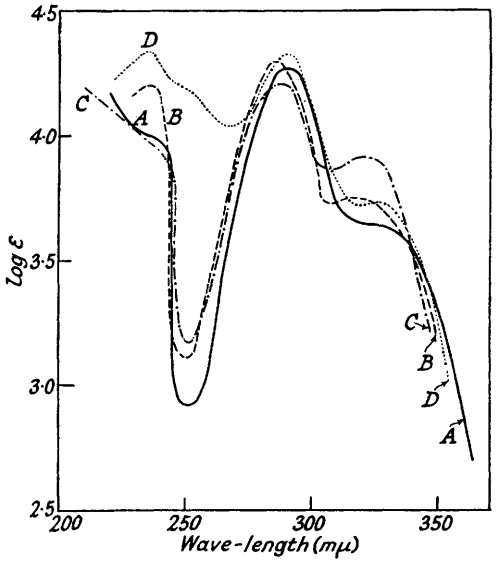


FIG. 1.

A, ——— *Acid* $C_{14}H_{15}O_7Cl$. *7-Chloro-2-hydroxy-4 : 6-dimethoxycoumaran-3-one-2-β-butyric acid.*

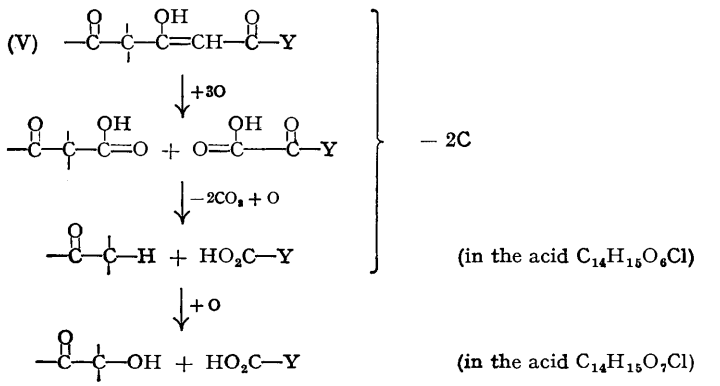
B, – – – – – *Acid* $C_{14}H_{15}O_6Cl$. *7-Chloro-4 : 6-dimethoxycoumaran-3-one-2-β-butyric acid.*

C, · — · — *Acid* $C_{13}H_{13}O_6Cl$. *7-Chloro-4(or 6)-hydroxy-6(or 4)-methoxycoumaran-3-one-2-β-butyric acid.*

D, · · · · · · · *Griseofulvin.*

(*All in methanol.*)

analogous conversion of griseofulvic acid into (II; R = H). In neither case could this fragment be traced as oxalic acid. The ultra-violet absorption curve of the acid (II; R = OH) closely resembles that of griseofulvin from 270 to 350 mμ (Fig. 1) and it is clear that the



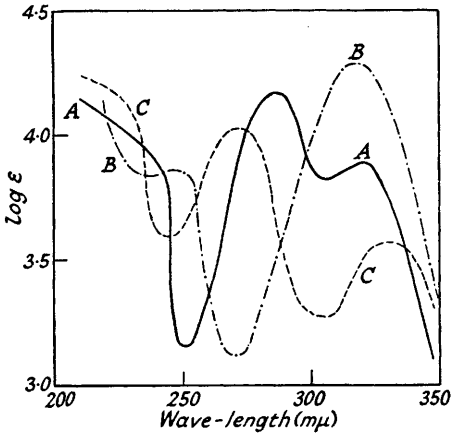
main chromophoric system of the latter compound is unaffected by the oxidation. It follows that the β-diketone grouping, believed to be present (Part I), must have undergone degradation and, if griseofulvic acid contained the partial structure (V), two molecules of

carbon dioxide could be lost—one each from the intermediate β-keto-acid and α-keto-acid groupings, for a total addition of 4 oxygen atoms, leaving a net loss of C_2.

Similar considerations apply to the oxidation of the dibasic norgriseofulvic acid to the dibasic acid $C_{13}H_{13}O_6Cl$. The ultra-violet absorption of the latter compound in methanol (Fig. 1) does not differ greatly from that of (II; R = OH) and it is clear that in this case also the β-diketone grouping undergoes oxidation. It also follows that the phenolic nucleus of norgriseofulvic acid (see Part I) is unaffected and that the second acid group in the compound $C_{13}H_{13}O_6Cl$ is the strongly acidic phenolic hydroxyl group believed to be present in norgriseofulvic acid.

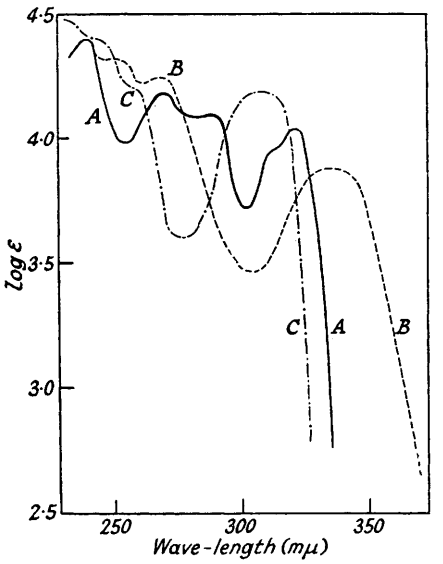
Like norgriseofulvic acid, the acid $C_{13}H_{13}O_6Cl$ shows some phenolic properties, and the ultra-violet absorption curve shows a marked shift to longer wave-lengths in 0·1N-sodium hydroxide (Fig. 2). Furthermore, the acid $C_{13}H_{13}O_6Cl$ can be remethylated with methyl

FIG. 2.



A, Acid $C_{13}H_{13}O_6Cl$ *in methanol. B, Acid* $C_{13}H_{13}O_6Cl$ *in* 0·1N-NaOH. *C, Acetate* $C_{15}H_{13}O_6Cl$ *in methanol.*

FIG. 3.

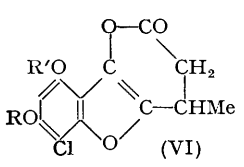


A, Phenol $C_{15}H_{13}O_4Cl$ *in methanol. B, Phenol* $C_{15}H_{13}O_4Cl$ *in* 0·1N-NaOH. *C,* 3 : 4 : 7-*Trimethoxydibenzofuran in methanol* (Tarbell, Frank, and Fanta, *J. Amer. Chem. Soc.*, 1946, **68**, 502).

sulphate to (II; R = H). The acid $C_{13}H_{13}O_6Cl$, in contrast to its methyl ether (II; R = H), is stable to alkaline permanganate at room temperature and an acid $C_{13}H_{13}O_7Cl$ corresponding to (II; R = OH) is not formed; nevertheless, permanganate oxidation of the diazomethane methylation product gives 3-chloro-2-hydroxy-4 : 6-dimethoxybenzoic acid (I).

An attempt is in progress to discover which of the two possible methyl ether groupings is split when (II; R = H) is hydrolysed to the acid $C_{13}H_{13}O_6Cl$.

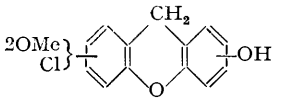
With acetic anhydride the acid $C_{13}H_{13}O_6Cl$ forms a neutral monoacetate $C_{15}H_{13}O_6Cl$ which titrates as a potential tribasic acid on lactone titration, regenerating the dibasic acid $C_{13}H_{13}O_6Cl$. It is considered to be (VI; R = Ac or Me, R′ = Me or Ac). This structure is supported by the ultra-violet absorption spectrum (Fig. 2) which indicates that the substituted coumaranone chromophore is no longer present and resembles the type of spectrum found with some compounds in which an ethylenic bond is conjugated with an aromatic nucleus [*e.g.*, the (—)-methoxyacetate of (±)-tetrahydrocannabinol (Russell *et al.*, *J.*, 1941, 826)]; the infra-red spectrum is in agreement with this view.



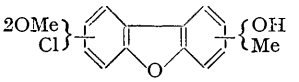
(VI)

Griseofulvin is stable to the action of potassium dichromate in acetic acid but is readily oxidised by chromic oxide to 3-methoxy-2 : 5-toluquinone (XI). This is not obtained when *iso*griseofulvin is oxidised with chromic oxide under the same conditions.

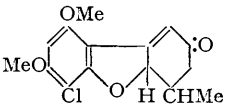
Griseofulvic acid is oxidised by alkaline yellow mercuric oxide to a dimethoxy-phenol, $C_{15}H_{13}O_4Cl$, the methyl ether of which is fully stable to permanganate in acetone and contains no ethylenic linkages on hydrogenation. The compound $C_{15}H_{13}O_4Cl$ is therefore aromatic and, accordingly, is optically inactive. It has no carbonyl reactivity and the infra-red spectrum confirms the absence of keto-groups. The fourth oxygen atom must therefore be present in an ether linkage and it follows that the compound contains three fused rings. Of the structures admissible from fundamental considerations, only the substituted dibenzopyran (VII) or dibenzofuran (VIII) need be considered and of these the ultra-violet absorption spectrum (Fig. 3) is consistent only with (VIII). Moreover, Kühn-Roth oxidations of the phenol $C_{15}H_{13}O_4Cl$ and its methyl ether show the presence of one *C*-methyl group, favouring (VIII).



(VII)



(VIII)

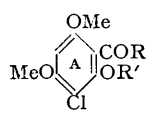


(IX)

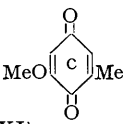
The compound $C_{15}H_{13}O_4Cl$ has, in fact, the aromatic system corresponding to the structure (IX) which Oxford *et al.* (*loc. cit.*) postulated for decarboxygriseofulvic acid, $C_{15}H_{15}O_4Cl$. As stated in Part I, the ultra-violet absorption of decarboxygriseofulvic acid is inconsistent with (IX); nevertheless, the close relation of the two substances has been confirmed by aerial oxidation of decarboxygriseofulvic acid in alkaline suspension to the phenol $C_{15}H_{13}O_4Cl$. This provides the explanation for our observation (Part I) that in the hydrolysis of griseofulvin and griseofulvic acid, good yields of decarboxygriseofulvic acid were obtained in a stream of nitrogen and less, or none, in a stream of air.

The mechanisms for the formation of decarboxygriseofulvic acid and the phenol, $C_{15}H_{13}O_4Cl$, from griseofulvic acid is discussed in Part IV (*J.*, 1952, 3977).

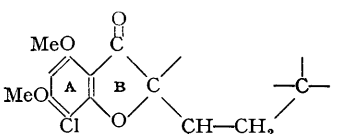
The constitution of the oxidation products makes it clear that griseofulvin contains an aromatic ring A (X) which carries the chlorine substituent and gives rise to the salicylic acid (I), and a second ring C which must be hydroaromatic and gives rise to the quinone (XI).



(X)



(XI)



(XII)

The carbon skeleton of griseofulvin has been shown (Part I) to be stable under acid conditions; it is unlikely, therefore, that any molecular rearrangement is involved in the formation of (XI). The similarity in the ultra-violet absorption of griseofulvin and 7-chloro-4 : 6-dimethoxycoumaranone-2-β-butyric acid also suggests that griseofulvin contains the substituted coumaranone ring system (XII).

Experimental

M. p.s are corrected. The infra-red spectra of solid samples were obtained as described in Part I. Microanalyses are by Drs. Strauss and Weiler, Oxford (who also determined some of the ultra-violet absorption spectra), by Mr. W. Brown, Imperial Chemical Industries Limited, Butterwick Research Laboratories, and by the Analytical Department, Imperial Chemical Industries Limited, Dyestuffs Division.

Permanganate Oxidation of Griseofulvin.—(a) *Potassium permanganate.* Griseofulvin (4 g.) was oxidised with potassium permanganate (16·0 g., 13·5 O) in the manner described by Oxford *et al.* (*loc. cit.*). 3-Chloro-2-hydroxy-4 : 6-dimethoxybenzoic acid was purified by sublimation at 160°/2 × 10^{-3} mm., followed by crystallisation from ethyl acetate, giving colourless needles

(104 mg.), m. p. 221—222° (decomp.) [Found: C, 46·5; H, 3·85; Cl, 14·65; OMe, 25·35%; *M* (Rast), 252. Calc. for $C_9H_9O_5Cl$: C, 46·45; H, 3·9; Cl, 15·25; 2OMe, 26·7%; *M*, 232·5]. The m. p. was found to vary between 200° and 222° (decomp.) depending on crystal size and rate of heating. It was confirmed that the acid, which gave a negative Millon but positive Gibbs reaction, was methylated by methanolic diazomethane to methyl 3-chloro-2 : 4 : 6-trimethoxybenzoate, identified by analysis and comparison with a synthetic specimen (Calam and Oxford, *J.*, 1939, 280).

7-Chloro-2-hydroxy-4 : 6-dimethoxycoumaranone-2-β-butyric acid (II; R = OH) was purified by crystallisation from ethyl methyl ketone–light petroleum (b. p. 60—80°) (charcoal), affording colourless needles (95 mg.), m. p. 198° (decomp.) (Found: C, 51·0; H, 4·7%; equiv., 323. Calc. for $C_{13}H_{14}O_5Cl{\cdot}CO_2H$: C, 50·8; H, 4·6%; equiv., 330·5). No volatile acids or saturated dibasic acids were detected in the ultimate acetone mother-liquors.

(b) *Zinc permanganate.* Griseofulvin (4·0 g.) in pure acetone (1300 ml.) was treated with a 1·9% aqueous solution of zinc permanganate (483 ml., 8O) at room temperature with shaking at 15-minutes' intervals. After 2·5 hours, the brown precipitate was collected and washed with acetone. The filtrate and washings were concentrated *in vacuo* to 300 ml. whereupon a colourless solid separated which was extracted with ether. The sodium carbonate-washed ethereal extract yielded unchanged griseofulvin (140 mg.); ether-extraction of the acidified carbonate washings gave the acid (II; R = OH) (1·1 g.), colourless needles, m. p. 178—186° (decomp.), from ethyl methyl ketone–light petroleum (b.p. 60—80°). The brown precipitate was worked up as before, yielding 3-chloro-2-hydroxy-4 : 6-dimethoxybenzoic acid (180 mg.) and the acid (II; R = OH) (100 mg.).

(c) *Search for volatile acids.* The aqueous acetone mother-liquors, from treatment of griseofulvin (4·0 g.) in acetone (1300 ml.) with zinc permanganate (7·0 g., 6O) in water (400 ml.), were made slightly alkaline by sodium carbonate and concentrated to 300 ml. Recovery of the precipitated solid by ether-extraction gave unchanged griseofulvin (200 mg.), m. p. and mixed m. p. 214—216°. Acidification of the sodium carbonate solution to Congo-red afforded 3-chloro-2-hydroxy-4 : 6-dimethoxybenzoic acid (40 mg.), m. p. 222—224° (decomp.). The acid mother-liquors were steam-distilled, and 50 ml. of distillate collected. Although the distillate required 3·00 ml. of 0·112N-sodium hydroxide for neutralisation (phenolphthalein), no volatile acids could be isolated.

Ether-extraction of the residual solution after steam-distillation, and recovery, gave the acid (II; R = OH) (1·1 g.), m. p. 185—189° after crystallisation from ethyl acetate.

Oxidation of Griseofulvic Acid with Potassium Permanganate.—A well-stirred solution of griseofulvic acid (1·0 g.) in sodium carbonate (20 ml.) was treated dropwise with 1% aqueous potassium permanganate until the pink colour persisted for 15 minutes (310 ml., 10O). After removal of manganese dioxide by filtration, the acidified mother-liquors were steam-distilled. The distillate required only 2·4 ml. of 0·1N-sodium hydroxide for neutralisation, and attempts to isolate volatile acids were unsuccessful. The residual solution from the steam-distlllation was concentrated to 200 ml. and extracted in turn as follows. (*a*) Ether (100 ml.) removed an amorphous solid which sublimed at 170°/10^{-4} mm. Dissolution of the yellow sublimate in ether, extraction with sodium hydrogen carbonate, and recovery afforded a colourless solid, crystallising from ethanol in needles (30 mg.), m. p. 200—210°, identified by the infra-red spectrum as lactone A (see below). The gum obtained by acidification of the bicarbonate extract was worked up with a similar fraction obtained in (*b*) below.

(*b*) Ethyl acetate (2 × 200 ml.) yielded a brown gum, which on sublimation at 10^{-4} mm. gave (i) a yellow gum at 140°, and (ii) a yellow solid at 170°. The former crystallised from ethanol in colourless needles (20 mg.), m. p. 218—220°, of lactone A (see below) (Found: C, 53·4; H, 4·4; Cl, 10·7. Calc. for $C_{14}H_{13}O_6Cl$: C, 53·7; H, 4·2; Cl, 11·3%), absorption max. in methanol at 328, 290, and ~236 mμ (log ε 3·61, 4·20, 4·20). Dissolution of fraction (ii) in ether and treatment as in (*a*) afforded the lactone A, m. p. 210—220°, and a yellow oil. The latter, combined with the corresponding fraction in (*a*), crystallised from ethyl methyl ketone–light petroleum (b. p. 40—60°) in colourless needles, m. p. 180° (decomp.), identified as the acid (II; R = OH) by the infra-red spectrum. (*c*) Continuous extraction (24 hours) with hot ethyl acetate, sublimation of the recovered material at 140°/10^{-4} mm., dissolution of the sublimate in ether, washing with water, and recovery afforded a sticky material from which a colourless solid (1 mg.), m. p. 160° (decomp.), was obtained on a porous plate. This acid differed from the acid (II; R = OH) by its greater solubility in water and by its dissolving to a colourless solution in bicarbonate.

Lactonisation of 7-Chloro-2-hydroxy-4 : 6-dimethoxycoumaranone-2-β-butyric Acid (II; R =

OH).—(a) *By sublimation.* Sublimation of the acid (100 mg.) at 180°/10^{-4} mm., followed by extraction of the sublimate with sodium carbonate (43 mg. recovery of acid), afforded a lactone, colourless needles (30 mg.), m. p. 218—222°, from ethanol. This lactone (now called lactone A) corresponds to the lactone, m. p. 222°, described by Oxford *et al.* (*loc. cit.*). Although neutral to litmus and Universal indicator in aqueous suspension, it slowly dissolved in sodium hydroxide and sodium hydrogen carbonate solutions, giving yellow solutions.

Lactone A appeared to be optically inactive, $[\alpha]_D^{18} \not> \pm 3°$ (*c*, 1·10 in acetone; *l*, 1 dm.). Treatment with excess of 0·1N-sodium hydroxide for 30 minutes at 100° and back-titration gave an apparent equivalent of 343 (Calc. for $C_{14}H_{13}O_6Cl$: 312·5). Acidification of the solution to Congo-red and recovery in ether gave lactone A, m. p. 218—220°, and not (II; R = OH).

(b) *With acetic anhydride.* Treatment of the acid (II; R = OH) (150 mg.) in dry pyridine (1·5 ml.) with acetic anhydride (0·6 ml.) at 35° for 4 days and pouring the reaction mixture into ice-water and dilute hydrochloric acid gave a colourless solid which was taken up in benzene and extracted with sodium carbonate solution. The dried benzene extract was chromatographed on alumina (5 × 0·5 cm.) (pH 4; activated at 150°). Elution of a band fluorescing vivid violet in ultra-violet light with benzene (15 ml.) afforded lactone B, $C_{14}H_{13}O_6Cl$, colourless needles (95 mg.) (from ethanol), m. p. 163°, which resolidified and then remelted at 178—180° (Found: C, 53·4; H, 4·2; Cl, 10·8; OMe, 19·2. $C_{14}H_{13}O_6Cl$ requires C, 53·7; H, 4·2; Cl, 11·3; 2OMe, 19·8%). The m. p. of lactone B was not raised by vacuum-sublimation followed by repeated crystallisation from ethanol. Seeding with lactone A yielded a small crop of crystals, m. p. 195°, not raised by further crystallisation. A mixture of lactones A and B melted indefinitely between 180—210°. Lactone B, like lactone A, was insoluble in cold sodium hydroxide and sodium carbonate but dissolved slowly when kept or warmed, giving yellow solutions from which the colour was discharged by acidification. The infra-red spectra of the two lactones were very similar but not completely identical; lactone A showed a band at 1150 cm.$^{-1}$ absent from the spectrum of lactone B. The latter showed a " shoulder " at 1125 cm.$^{-1}$ absent from the former.

(c) *With sulphuric acid.* The orange solution obtained on keeping the acid (50 mg.) overnight in concentrated sulphuric acid (0·5 ml.) was poured over crushed ice. The yellow solid (37 mg.), after being washed with sodium carbonate and water, was sublimed at 190°/10^{-4} mm. and the yellow sublimate crystallised from ethanol. Colourless needles, m. p. and mixed m. p. with lactone B, 180—182°, were separated by hand-picking from colourless needles, m. p. 182—190°, which are considered to be a mixture of lactones A and B.

Permanganate Oxidation of Decarboxygriseofulvic Acid.—The brown precipitate, obtained by treatment of decarboxygriseofulvic acid (500 mg.) in pure acetone (10 ml.) with potassium permanganate (2·0 g.) in acetone (100 ml.) for 5 hours, was ground with dilute aqueous ammonia and worked up as described by Oxford *et al.* The product crystallised in needles (175 mg.), m. p. 196° (decomp.), from ethyl acetate after sublimation at 160°/10^{-3} mm. and was identified as 3-chloro-2-hydroxy-4 : 6-dimethoxybenzoic acid by mixed m. p., infra-red spectrum, and *X*-ray diffraction pattern.

Potassium Permanganate Oxidation of the Acid (II; R = OH).—The acid (100 mg.) in acetone (5 ml.) was treated with potassium permanganate (400 mg.) in acetone (20 ml.) and kept for 24 hours at room temperature with occasional shaking. The brown precipitate was worked up in the usual way, affording 3-chloro-2-hydroxy-4 : 6-dimethoxybenzoic acid (50 mg.), m. p. 218—222°, identified by mixed m. p., infra-red absorption spectrum, and *X*-ray powder diffraction. The aqueous acid filtrate slowly deposited unchanged starting material (20 mg.), m. p. and mixed m. p., 188—190° (decomp.).

Oxidation of Griseofulvic Acid with Hydrogen Peroxide.—Griseofulvic acid (1·0 g.) in 0·1N-sodium hydroxide (125 ml.) was treated with 30% hydrogen peroxide (25 ml.) and, after 2 days at room temperature with occasional stirring, a further 20 ml. of 0·1N-sodium hydroxide were added. At the end of the fourth day, the solid obtained by acidification was extracted (10 minutes) with boiling toluene, leaving unchanged griseofulvic acid, m. p. 240—244° (decomp.) (270 mg.). Concentration of the toluene extract furnished 7-*chloro*-4 : 6-*dimethoxycoumaranone*-2-β-*butyric acid* (II; R = H) which crystallised from toluene in colourless prisms (500 mg.), m. p. 152—154°, $[\alpha]_D^{20}$ −38·7° (*c*, 1·627 in methanol) (Found: C, 53·9, 54·05; H, 4·95, 4·9; Cl, 11·1, 10·5; OMe, 19·05, 18·65%; equiv., 320. $C_{13}H_{14}O_4Cl{\cdot}CO_2H$ requires C, 53·45; H, 4·75; Cl, 11·3; 2OMe, 19·7%; equiv., 314·5), ultra-violet absorption max. in methanol at 321, 286, and 236 mμ (log ε 3·73, 4·33, 4·23). The acid (II; R = H) showed no reducing properties, did not form a dinitrophenylhydrazone with 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid, and reacted only slowly with bromine in carbon tetrachloride. It gave no colour with ferric chloride, and the Millon reaction was negative.

Permanganate Oxidation of 7-Chloro-4 : 6-dimethoxycoumaranone-2-β-butyric Acid (II; R = H).—To an ice-cold solution of the acid (160 mg.) in N-sodium carbonate (10 ml.) a solution of potassium permanganate (0·455 g.) in water (10 ml.) was added dropwise and with stirring, during 1½ hours, a permanent pink colour being then obtained (3—4 ml., 3O). Decolorisation with sulphur dioxide and acidification left a crystalline solid (40 mg.) which was combined with a further fraction (40 mg.), obtained on concentration of the mother-liquors *in vacuo*, and crystallised from ethyl methyl ketone–light petroleum (b. p. 60—80°); it had m. p. and mixed m. p. with (II; R = OH), 204·5° (decomp.).

Alkaline Hydrolysis of 7-Chloro-4 : 6-dimethoxycoumaranone-2-β-butyric Acid.—The acid (350 mg.) in 3N-sodium hydroxide (10 ml.) was heated under reflux for 5 hours in a slow stream of nitrogen. The acidified solution, on being kept for 24 hours at 0°, deposited a colourless solid (200 mg.), m. p. 194—198° (decomp.). Recrystallisation twice from ethyl acetate afforded plates, m. p. 210·5—214° (decomp.), of 7-chloro-4(or 6)-hydroxy-6(or 4)-methoxycoumaranone-2-β-butyric acid, $[\alpha]_D^{18}$ −71° (*c*, 1·10 in methanol) [Found: C, 51·65, 51·65; H, 4·45, 4·5; Cl, 11·55; OMe, 10·4%; equiv., 162·5; $C_{13}H_{13}O_6Cl$ requires C, 51·9; H, 4·3; Cl, 11·8; 1OMe, 10·3%; equiv., 150 (dibasic acid)]. Ultra-violet absorption max.: 321, 289, ~242 mμ (log ε 3·91, 4·20, 3·92) in methanol; 320, 250 mμ (log ε 4·32, 3·89) in 0·1N-sodium hydroxide. The dibasic acid $C_{13}H_{13}O_6Cl$ gave a positive Millon reaction and a red-brown colour with ferric chloride and showed weak but positive coupling with diazotised amines. It showed no reducing properties and did not react with 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid.

Methylation of the acid $C_{13}H_{13}O_6Cl$ (30 mg.) with methyl sulphate and 10% sodium hydroxide in the usual way afforded a white solid which crystallised from toluene in prisms, m. p. 146—149° (7 mg.), identified as the acid (II; R = H) by comparison of the infra-red spectra.

The acid $C_{13}H_{13}O_6Cl$ (100 mg.) and acetic anhydride (1·5 ml.) were heated under reflux for 30 minutes and the cooled solution was poured on crushed ice. The resultant neutral *monoacetyl* compound crystallised from ethanol in needles, (70 mg.) m. p. 197—198° [Found: C, 55·6, 55·8; H, 4·2, 4·1; Cl, 10·6; OMe, 10·6; Ac, 18·8%; *M* (Rast), 292. $C_{15}H_{13}O_6Cl$ requires C, 55·5; H, 4·0; Cl, 10·9; 1OMe, 9·6; 1Ac, 13·3%; *M*, 324). Ultra-violet absorption max: 332, 272, ~229 mμ (log ε 3·62, 4·07, 4·19) in methanol. The infra-red spectrum showed a single intense band in the >C=O region at 1774 cm.$^{-1}$ and a weak band at 1652 cm.$^{-1}$ attributed to the ethylenic bond. The 1774 cm.$^{-1}$ band is consistent with the presence of phenyl or vinyl ester (or lactone) groupings (Grove and Willis, *J.*, 1951, 877; Grove, *J.*, 1951, 883) and its strength explained by the presence of two such groupings in the molecule. The acetate which showed an intense fluorescence in ultra-violet light was insoluble in potassium hydrogen carbonate solution but dissolved slowly in warm sodium hydroxide.

The acetate (30·5 mg.) was heated on a water-bath for 45 minutes with 0·121N-sodium hydroxide (5·80 ml.). Back-titration with 0·121N-hydrochloric acid required 3·80 ml., giving an apparent equivalent of 105 ($C_{15}H_{13}O_6Cl$ requires equiv. 108 as a potential tribasic acid). The solution, made strongly acid with concentrated hydrochloric acid and concentrated, yielded the dibasic acid $C_{13}H_{13}O_6Cl$, identified by mixed m. p. and infra-red spectrum.

The acetate gave no colour with ferric chloride and a negative Millon reaction. When heated with an equal weight of *p*-toluidine for 1 hour on the steam-bath it gave the *monotoluidide*, m. p. 223—225° (from aqueous methanol), of the acid $C_{13}H_{13}O_6Cl$ (Found: C, 61·95; H, 5·2; N, 4·2; Cl, 8·6. $C_{20}H_{20}O_5NCl$ requires C, 61·6; H, 5·15; N, 3·6; Cl, 9·1%). This was insoluble in cold aqueous sodium hydrogen carbonate and sodium carbonate, but soluble in sodium hydroxide solution.

Oxidation of Norgriseofulvic Acid to the Acid $C_{13}H_{13}O_6Cl$.—(a) *Hydrogen peroxide.* Norgriseofulvic acid (400 mg.) in N-potassium hydroxide (5·2 ml.) and water (20·8 ml.) was treated with 30% hydrogen peroxide (5 ml.). After 24 hours the faintly purple solution was acidified and cooled in ice; the deposited solid crystallised from ethyl acetate in colourless plates (130 mg.), m. p. and mixed m. p. with the dibasic acid, $C_{13}H_{13}O_6Cl$, 211—213°.

(b) *Potassium permanganate.* To a well-stirred solution of norgriseofulvic acid (250 mg.) in N-sodium carbonate (15 ml.) at 0°, a solution of potassium permanganate (0·081 g./ml., 1O/ml.) was added dropwise until oxidation was complete (10—11 ml.). Decolorisation with sulphur dioxide, followed by acidification, extraction with ether, and recovery, gave a gum crystallising in prisms (from benzene–ethyl acetate), m. p. 202—206° (decomp.), identified as the acid $C_{13}H_{13}O_6Cl$ by mixed m. p. and comparison of the infra-red spectra.

Oxidation of Griseofulvic Acid with Yellow Mercuric Oxide.—A mixture of griseofulvic acid (1·8 g.), 2N-sodium hydroxide (5·25 ml.), water (12·9 ml.), and yellow mercuric oxide (2·5 g.) was stirred on a steam-bath for 18 hours (rapid darkening and finally production of some

metallic mercury). To the grey suspension was added saturated sodium chloride solution (25 ml.), and the precipitate removed (centrifuge; washing with water). Acidification of the alkaline mother-liquors gave unchanged griseofulvic acid (500 mg.), m. p. 226—228°. The dark precipitate, suspended in N-hydrochloric acid (10 ml.), was treated with hydrogen sulphide for 7 hours at 50—60° and thereafter for 11 hours at room temperature. Extraction (Soxhlet) of the washed precipitate with methanol furnished a brown solid which crystallised (charcoal) from toluene in fine colourless needles (500 mg.), m. p. 199° [Found: C, 61·5; H, 4·75; Cl, 12·45; OMe, 20·65; *C*-Me (Kühn–Roth), 6·4. $C_{15}H_{13}O_4Cl$ requires C, 61·5; H, 4·5; Cl, 12·3; 2OMe, 21·2; 1*C*-Me, 5·1%]. Ultra-violet absorption max.: 320, 310, 286, 268, 238 mμ (log ε 4·04, 3·94, 4·10, 4·18, 4·40) in methanol; 335, 268, 252 mμ (log ε 3·88, 4·28, 4·32) in 0·1N-sodium hydroxide. The infra-red spectrum showed a strong –OH absorption at 3255 cm.$^{-1}$ and the absence of carbonyl groups. The *phenol*, which was optically inactive, dissolved in hot 2N-sodium hydroxide from which the sodium salt crystallised on cooling. The phenol coupled readily with diazonium salts although both Millon and Gibbs reactions were negative. It was unsaturated to potassium permanganate and absorbed bromine in chloroform solution with evolution of hydrobromic acid. The *picrate* formed brick-red needles, m. p. 196—198°, from aqueous ethanol (Found: C, 47·95; H, 3·2; N, 8·7; Cl, 7·4. $C_{21}H_{16}O_{11}N_3Cl$ requires C, 47·8; H, 3·05; N, 8·05; Cl, 6·8%); the *toluene*-p-*sulphonyl* derivative crystallised in colourless prisms, m. p. 196°, from ethyl acetate (Found: C, 58·1; H, 4·15: $C_{22}H_{19}O_6ClS$ requires C, 59·1; H, 4·25%).

The phenol was also formed by oxidation of griseofulvin by alkaline yellow mercuric oxide.

The *methyl ether*, obtained by treatment with methyl sulphate and alkali, crystallised in flat needles from ethanol (charcoal), and hexagonal prisms from aqueous acetone, and had m. p. 168—170° (Found: C, 62·35; H, 4·85; OMe, 29·9; *C*-Me, 5·9. $C_{16}H_{15}O_4Cl$ requires C, 62·65; H, 4·9; 3OMe, 30·35; 1*C*-Me, 4·9%.) It was optically inactive and was stable to permanganate in acetone.

Chromic Oxide Oxidations.—(a) *Griseofulvin.* Griseofulvin (1·0 g.) in warm acetic acid (15 ml.) was treated dropwise with chromic oxide (3·0 g.) in acetic acid (15 ml.) and water (6 ml.), whereupon a vigorous reaction set in. After spontaneous refluxing (10 minutes), the solution was heated under reflux for 30 minutes, diluted with water, and extracted with benzene. Recovery and sublimation at 90°/10^{-4} mm. gave 3-methoxy-2 : 5-toluquinone, long yellow needles (130 mg.), m. p. 148—150°, from methanol, identified by analysis and mixed m. p. with a specimen synthesised as described by Henrich and Nachtigall (*Ber.*, 1903, **36**, 889). Crystallisation of the residue from ethanol gave unchanged griseofulvin (34 mg.), m. p. 217—218°.

(b) iso*Griseofulvin.* Oxidation as in (*a*) gave a small amount of yellow intractable oil, devoid of ketonic properties and partly soluble in sodium hydroxide, and a little unchanged *iso*griseofulvin.

(c) *Griseofulvic acid.* This (250 mg.) was oxidised with chromium trioxide (750 mg.) as in (*a*). Benzene extraction of the diluted reaction mixture yielded a gum which crystallised from methanol in colourless needles, m. p. 194—195°, identified as lactone A of the acid $C_{14}H_{15}O_7Cl$ by mixed m. p. and infra-red spectroscopy. The m. p. could not be raised by crystallisation or sublimation.

Hypobromite Oxidation of Griseofulvic Acid.—Griseofulvic acid (160 mg.) in 2·5 N-sodium hydroxide (2 ml.) was treated with 2 ml. of a solution containing 6·5 g. of bromine in 35 ml. of solution. After 1 hour, the liberated bromoform was separated and excess of hypobromite removed with sulphur dioxide. Acidification afforded an orange gum which solidified during 24 hours at 0°. After filtration and extraction with toluene, the toluene-insoluble fraction (35 mg.) was crystallised from ethyl methyl ketone–light petroleum in colourless needles, m. p. 180° (decomp.), identified as the acid (II; R = OH) by a mixed m. p. and infra-red absorption spectrum. Evaporation of the toluene left a gummy solid which was intractable, but which, from its behaviour, probably contained some of the acid (II; R = H).

Aerial Oxidation of Decarboxygriseofulvic Acid.—Decarboxygriseofulvic acid (110 mg.) and 2N-sodium hydroxide (17 ml.) were heated under reflux while a slow stream of air was passed through the solution. At the end of 3 hours some solid material remained undissolved. Crystalline material which separated on cooling was dissolved by the addition of water, and the solution filtered. Concentration of the filtrate *in vacuo* and dilution with an equal volume of a saturated solution of sodium chloride afforded a crystalline solid which was collected (centrifuge) and dissolved in water. Acidification of the aqueous solution yielded a solid which crystallised from toluene in long felted needles, m. p. 198—201° (8 mg.), identified as the phenol $C_{15}H_{13}O_4Cl$ by comparison of the infra-red and ultra-violet absorption spectra.

Oxidation of the Acid $C_{13}H_{13}O_6Cl$.—(a) The acid $C_{13}H_{13}O_6Cl$ (120 mg.) in 2N-sodium carbonate (1 ml.) and water (5 ml.) was recovered unchanged after treatment with potassium permanganate (1·0 g.) in water (25 ml.) at room temperature.

(b) *After methylation.* The acid (400 mg.) in methanol (10 ml.) was treated with excess of ethereal diazomethane and the product, without purification, was directly oxidised by heating its acetone solution (40 ml.) under reflux for 16 hours with powdered potassium permanganate. The resultant solid was shaken with 2N-ammonia solution for 2 hours. Acidification of the filtrate gave 3-chloro-2-hydroxy-4 : 6-dimethoxybenzoic acid (100 mg.), m. p. 206—208°, identified by comparison of the *X*-ray diffraction patterns.

We are indebted to Mr. H. A. Willis, Imperial Chemical Industries Limited, Plastics Division, for infra-red spectrographic facilities and to Dr. A. Spinks and Mr. M. St. C. Flett who obtained some of the ultra-violet and infra-red spectra.

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