Studies in the Biochemistry of Micro-organisms

107. METABOLITES OF *PENICILLIUM VIRIDICATUM* WESTLING: VIRIDICATIC ACID (ETHYLCARLOSIC ACID)*

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The mould *Penicillium viridicatum* Westling produces several crystalline metabolites. The mycelium has afforded viridicatin, $C_{15}H_{11}O_2N$, which was first isolated and described by Cunningham & Freeman (1953). This product was later obtained from *P. cyclopium* Westling by Bracken, Pocker & Raistrick (1954), who determined the molecular structure as 2:3-dihydroxy-4-phenylquinoline or its ketotautomer. A product closely related to viridicatin, namely cyclopenin, $C_{17}H_{14}O_3N_2$, was also obtained from the mycelium of *P. cyclopium*, although not from the strains producing viridicatin. On mild acid hydrolysis, cyclopenin yields viridicatin, methylamine and carbon dioxide; on the basis of this reaction Bracken *et al.* (1954) suggested for cyclopenin two alternative structures.

The culture fluid of *P. viridicatum* has also afforded metabolic products, namely mycophenolic acid (Burton, 1949), originally obtained from *P. brevicompactum* Dierckx, and cyclopolic acid and cyclopaldic acid (Sankhala, 1957), originally described as metabolic products of *P. cyclopium* (Birkinshaw, Raistrick, Ross & Stickings, 1952).

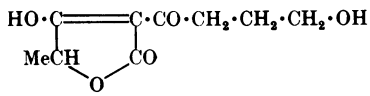
This paper mainly concerns further metabolites of *P. viridicatum* derived from the culture fluid. Two products have been isolated; one of these, terrestric acid, is known and was previously obtained from the culture solution of *P. terrestre* Jensen by Birkinshaw & Raistrick (1936), who established the structure as that of an ethylcarolic acid. It is thus a tetronic acid derivative. The second product now obtained from the culture fluid of *P. viridicatum* is a hitherto undescribed acidic metabolite for which we propose the name viridicatic acid. This is shown to have the structure of an ethylcarlosic acid and is therefore also a tetronic acid derivative. From the mycelium the known and frequently encountered fungal products mannitol, *i*-erythritol and ergosteryl palmitate were isolated in addition to viridicatin.

These observations invite examination of the morphological relationships between these species of *Penicillium* showing biochemical similarities. *P. viridicatum* and *P. cyclopium* both belong to the group Asymmetrica–Fasciculata and Smith (1946) considers that all the blue–green and yellow–green strains with similar morphology should be grouped together as the *P. cyclopium–viridicatum* series. *P. terrestre* is actually placed in the Asymmetrica–Funiculosa, but Raper & Thom (1949, p. 445) consider that the general colony aspect and the size and pattern of the penicilli of the *P. terrestre* series closely approach those of the Lanata on the one hand and the Fasciculata on the other. *P. brevicompactum* is assigned to the group Asymmetrica–Velutina but a culture maintained as NRRL 863 showed characteristics relating it to the *P. terrestre* series (Raper & Thom, 1949, p. 410).

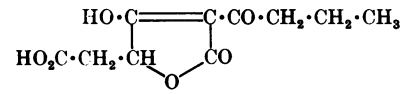
On the other hand, since terrestric acid and viridicatic acid are tetronic acid derivatives, some kinship might be expected between the species considered above and *P. charlesii* G. Smith, which was the original source of several tetronic acids (Clutterbuck, Haworth, Raistrick, Smith & Stacey, 1934). Actually *P. charlesii* is assigned to the group Monoverticillata–Ramigena, which is widely separated from the Asymmetrica.

The fungal strain employed in this investigation was isolated from Italian garden soil and was identified by Mr G. Smith as *P. viridicatum* Westling. When grown on Czapek–Dox medium the culture filtrate gave a moderate brown colour with ferric chloride, gave an orange precipitate with Brady's reagent on keeping and decolorized bromine water. When the acidified filtrate was extracted with ether, crystalline material was obtained. This material on fractionation yielded two acidic products, one of which was identified as terrestric acid (ethylcarolic acid), and α-substituted γ-methyltetronic acid (II). The second product from the culture fluid proved to be a hitherto undescribed acidic substance, a colourless crystalline acid, $C_{12}H_{16}O_6$, herein named viridicatic acid, having properties similar to the tetronic acids and in particular to carlosic acid (III) (Clutterbuck, Raistrick & Reuter, 1935). This similarity is evident in the following summary of the properties of viridicatic acid (IV).

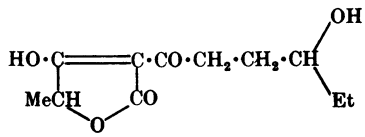
* Part 106: Stickings (1959).



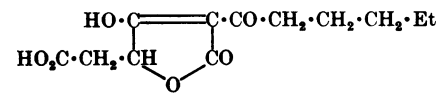
(I) Carolic acid (hydrated form)



(III) Carlosic acid



(II) Terrestric acid (hydrated form)



(IV) Viridicatic acid

(1) It gives with aqueous ferric choride an immediate brownish-yellow precipitate, insoluble in excess of reagent. Like carlosic acid it gives no colour with sodium nitrite.

(2) The acid titrates as dibasic and like carlosic acid shows a negative optical rotation.

(3) On short acid hydrolysis it affords carbon dioxide, *n*-hexanoic acid and β-hydroxylaevulic acid (isolated as 2:4-dinitrophenylhydrazone), which breaks down further on prolonged hydrolysis to afford a second mol. of carbon dioxide and acetoin. Under similar prolonged hydrolysis carlosic acid yields 2 mol. of carbon dioxide and 1 mol. each of *n*-butyric acid and acetoin.

(4) On bromination in aqueous 50 % acetic acid it gives (−)-α-bromo-γ-carboxymethyltetronic acid identical with that obtained from carlosic acid.

(5) The absorption spectrum (in ethanol) shows peaks in the ultraviolet at λ 230 mμ (log ε 3·86) and 268 mμ (log ε 4·10), which is consistent with its being a tetronic acid derivative. Thus carlosic acid (in water) shows maxima at 230 mμ (log ε 4·12) and 265 mμ (log ε 4·15) (Herbert & Hirst, 1935).

(6) On catalytic reduction (palladium on charcoal) 2 mol. of hydrogen are absorbed and a crystalline acid, $C_{12}H_{18}O_5$, is formed. This is consistent with the reduction of a CO group to a CH_2 group, such as occurs in the reduction of other α-acyltetronic acids (e.g. carlosic acid to the so-called tetrahydrocarlosic acid). This interpretation of the reaction is confirmed by the absorption spectra. The ultraviolet now shows only a single band at λ 230 mμ (log ε 4·08) in ethanol [cf. tetrahydrocarlosic acid, having a single band at λ 252 mμ (log ε 3·95) in water]. The infrared bands of viridicatic acid at 1605, 1577 (due to conjugation) and 1745 $cm.^{-1}$ (due to carbonyl) disappear on reduction.

It thus appears that viridicatic acid has the structure (−)-α-hexanoyl-γ-carboxymethyltetronic acid (IV), which differs from carlosic acid (III) only in the replacement at the α-carbon of the butyryl by the *n*-hexanoyl residue. The relationship between viridicatic acid (IV) and carlosic acid (III) is similar to that between terrestric acid (II) and carolic acid (I) since the second member of each pair differs from the first member only in the additional ethyl group replacing hydrogen on the ω-carbon atom of the α-substituent of the tetronic acid. It is to be noted, however, that the ω-carbon atom of carolic acid (hydrated form) carries a hydroxy group. The introduction of the ethyl group therefore creates a new centre of asymmetry; this does not happen in the transition from carlosic acid to viridicatic acid.

Because of the close relationship between these two pairs of acids, the isolation of terrestric acid together with viridicatic acid as metabolites of one particular strain of *P. viridicatum* suggests that the additional ethyl groups present in these acids arise by the incorporation of an additional acetate residue into the lower homologue or its precursor in each case, the acetate grouping being reduced in the further course of biosynthesis.

Lybing & Reio (1958) have clearly demonstrated the regularly alternating high and low radioactivity in the carbon atoms of the α-side chain of carolic acid and carlosic acid obtained by the agency of *Penicillium charlesii* from $CH_3 \cdot {}^{14}CO_2H$. This indicates that the chain is formed from acetate units. An addition of one acetate unit only would be required to produce the chains required for terrestric acid and viridicatic acid respectively.

EXPERIMENTAL

Combustion analyses. These were carried out by Dr Ing. A. Schoeller. All melting points are corrected.

Organism. The culture employed was isolated from a sample of Italian garden soil by Mr G. Agosti in 1956 and was identified by Mr G. Smith as *Penicillium viridicatum* Westling. It bears the L.S.H.T.M. catalogue no. G.A. 834.

Cultural conditions. The culture medium used was a Czapek–Dox medium of the following composition: glucose, 50 g.; $NaNO_3$, 2 g.; KH_2PO_4, 1 g.; KCl, 0·5 g.; $MgSO_4$, $7H_2O$, 0·5 g.; $FeSO_4,7H_2O$, 0·01 g.; water to 1 l. This was distributed in 350 ml. amounts in 1 l. conical flasks, sterilized by steaming on 3 successive days, sown with a suspension of spores of the culture grown on wort–agar slopes and incubated at 24° in the dark.

Optimum time of incubation. In preliminary experiments three flasks were harvested after 7, 14 and 21 days and the culture filtrates were extracted with ether. On evaporation of the ether, crystalline material was obtained after 7 days'

Table 1. *Reactions of culture filtrates of* Penicillium viridicatum

Period of growth (days)	pH	Residual glucose (%)	Bromine water used by 5 ml. (ml.)	$FeCl_3$ reaction	Time after Brady's reagent orange ppt. (hr.)
5	4·0	3·66	0·25	Dark yellow	4–5
7	3·7	2·70	0·40	Dark yellow	4–5
9	3·5	2·02	0·50	Dark yellow	3–4
12	3·5	1·67	0·60	Light brown	3–4
15	3·5	1·08	0·85	Light brown	2–3

Table 2. *Yield of metabolic products extracted with ether from culture filtrates of 20 flasks of* Penicillium viridicatum

	pH 3·5			pH 2·0		
Period of growth (days)	Total gummy material (g.)	Crystalline product (g.)	(m.p.)	Total gummy material (g.)	Crystalline product (g.)	(m.p.)
5	0·39	0·05	168–170°	2·0	0·15	170–172°
7	0·80	0·07	165–170	3·8	0·58	168–170
9	0·95	Nil	—	5·5	0·37	160–170
12	1·45	Nil	—	6·5	0·33	160–170
15	1·85	Nil	—	8·8	0·34	160–168

incubation but only intractable gummy material from the culture filtrates after 14 and 21 days. The crystalline material, of m.p. 165–170°, was mainly the new metabolic product. To determine the optimum period of incubation for maximum yield of the new product, 100 flasks of the culture medium were inoculated and 20 flasks were harvested at the end of 5, 7, 9, 12 and 15 days. The culture filtrates were examined for pH, residual glucose and for reactions with aq. $FeCl_3$, Brady's reagent and bromine water. The pH was determined by means of indicator papers, residual glucose by polarimeter and the reactions with Brady's reagent were performed by adding 5 ml. of the reagent to 5 ml. of the culture filtrate. For the bromine absorption, saturated bromine water was added drop by drop to 5 ml. of the culture filtrate, until the bromine colour persisted. The results of these tests are recorded in Table 1.

The culture filtrates were then concentrated under reduced pressure at 30–35° to about 750 ml. This volume was extracted four times with 0·5 vol. of ether, first at the original pH 3·5, then again after adjustment to pH 2·0 with conc. HCl. The amount of gummy extract and the weight of crystalline material separated therefrom by trituration with ether are recorded in Table 2. The maximum yield of crystalline material was thus obtainable after 7 days' incubation followed by extraction at pH 2·0.

Isolation and purification of metabolic products. Batches of 100 flasks were sown and harvested after 7 days. The culture filtrate of each batch was concentrated to about 1·5 l., adjusted to pH 2·0 with conc. HCl and extracted with ether (4 × 750 ml.). The combined ether extracts were concentrated to 500 ml. The acidic fraction was extracted from the ether with saturated aq. $NaHCO_3$, and the latter was acidified with HCl and the product transferred to ether. The solution was dried over anhydrous Na_2SO_4 and concentrated to 25–30 ml. On keeping, the solution deposited gummy crystals (11·2 g.) which, after trituration with ether, gave a yellowish solid. Three recrystallizations from ethyl acetate afforded colourless platelets (2·01 g.) of viridicatic acid of constant m.p. 174·5°.

The ether mother liquor and washings from the viridicatic acid were combined, diluted to 100 ml. with boiling ether and added to 500 ml. of boiling light petroleum (40–60°). The mixture, kept overnight at 0–5°, deposited a crystalline solid, m.p. 85–87° (1·97 g.). A further crop (1·03 g.) of m.p. 83–85° was obtained on concentration of the mother liquor. The total crude material was purified by recrystallization from light petroleum (60–80°) and was obtained as colourless needles (2·1 g.) of m.p. 89°, not depressed on admixture with an authentic sample of terrestric acid; $[\alpha]_{5461}^{20} + 59·5°$ in water (*c*, 1) [Found: C, 62·7; H, 6·8; equivalent (phenolphthalein), 211. Calc. for $C_{11}H_{14}O_4$: C, 62·8; H, 6·7%; mol.wt. 210]. The product gave an orange colour with aq. $FeCl_3$ and a faint violet with $NaNO_2$ on keeping overnight. These reactions are given by terrestric acid, which has $[\alpha]_{5461}^{20} + 61·1°$ in water (*c*, 0·5) (Birkinshaw & Raistrick, 1936). The part of the ether extract insoluble in aq. $NaHCO_3$, on removal of solvent, was a tarry material from which no crystalline product could be obtained.

Mycelial products. The dried mycelium (268 g.) was ground and extracted continuously first with light petroleum (b.p. 40–60°) and then with ethyl ether. Two colourless products were obtained from the light petroleum extracts, namely (i) viridicatin (0·26 g.), identified by mixed m.p. with authentic viridicatin and by preparation of the diacetyl derivative, and (ii) ergosteryl palmitate (0·65 g.) identified by Liebermann–Burchard colour reaction and by m.p. and by mixed m.p. with an authentic sample. From the ether only *i*-erythritol (2·65 g.) was obtained, identified by mixed m.p. with an authentic sample and by preparation of the tetra-acetyl derivative. Another batch of mycelium (250 g.), when extracted with ethyl acetate after removing from it all light petroleum-soluble material, yielded colourless needles of m.p. 165–166° (1·05 g.), identified as mannitol by mixed m.p. and preparation of the tribenzylidene derivative.

Properties of viridicatic acid. The acid crystallizes in colourless platelets of m.p. 174·5°, resetting on cooling and

remelting at the same temperature. It sublimes unchanged in high vacuum at 140–150°; $[\alpha]_{5461}^{20}$ – 105° in ethanol (*c*, 1), light-absorption values in ethanol $\lambda_{max.}$ 230, 268 mμ, log ε 3·86, 4·10 respectively [Found: C, 56·4, 56·1; H, 6·2, 6·3; OMe, nil; equivalent (phenolphthalein) 128. Calc. for $C_{12}H_{16}O_6$: C, 56·3; H, 6·25%; mol.wt. 256].

Viridicatic acid gives with aq. $FeCl_3$ an immediate brownish ppt. insoluble in excess of the reagent but gives no colour with aq. $NaNO_2$. It does not reduce ammoniacal $AgNO_3$ or Fehling's solution. It gives no colour with sodium nitroprusside solution but gives a violet with *m*-dinitrobenzene and 3:5-dinitrobenzoic acid in excess of dilute aq. NaOH (test for: $\cdot CH_2 \cdot CO \cdot$, $\cdot CH{:}C(OH) \cdot$, Mann & Saunders, 1944). It gives no iodoform reaction and does not restore the colour of Schiff's reagent.

Derivatives of viridicatic acid

Viridicatic acid failed to give acetyl or benzoyl derivatives by the usual methods; this behaviour is similar to that shown by carlosic acid.

Mono-p-bromophenacyl ester. Viridicatic acid (0·118 g.) was neutralized with 0·1 N-NaOH (8·96 ml.) and boiled under reflux with *p*-bromophenacyl bromide (0·25 g.) and ethanol (15 ml.). The crude ester (0·21 g., m.p. 185–188°) was recrystallized from ethanol and the *mono ester* was obtained as colourless rods of m.p. 188·5° [Found: C, 52·6; H, 4·6; Br, 17·9; $C_{20}H_{21}O_7Br$ requires C, 52·9; H, 4·8; Br, 17·8%].

Semicarbazone. Viridicatic acid (0·05 g.), semicarbazide hydrochloride (0·026 g.) and sodium acetate (0·023 g.) were dissolved in water (25 ml.) at 90°. The product (0·053 g., m.p. 197–198°) was collected and recrystallized from hot water, thus affording needles of the *semicarbazone*, m.p. 198–199° [Found: C, 49·5; H, 6·0; N, 13·7. $C_{13}H_{19}O_6N_3$ requires C, 49·8; H, 6·1; N, 13·4%].

Reaction with diazomethane. Viridicatic acid (0·116 g.) was suspended in ether and excess of diazomethane in ether was added. The reaction appeared complete in a few minutes. After 4–5 hr. the ether was removed and the residue was dried over H_2SO_4 *in vacuo* for several days. As with carlosic acid a yellowish oil (principally the *dimethyl derivative*) resulted, which did not crystallize [Found: OMe, 22·9; $C_{12}H_{14}O_4(OMe)_2$ requires 21·8%]. The methoxyl content, which was somewhat high for two methoxy groups, as with the corresponding derivative of carlosic acid, suggests that there may have been slight enolization (followed by methylation) in the α-acyl side chain.

Catalytic hydrogenation of viridicatic acid. Viridicatic acid (0·5 g.) dissolved in ethanol (100 ml.) was hydrogenated in the presence of a palladium on charcoal catalyst (4 g.) added as suspension in ethanol (60 ml.). The uptake of hydrogen amounted to 2 mol. after about ½ hr. The catalyst was removed by filtration and the filtrate was evaporated to dryness. The yellowish-white solid obtained, after repeated recrystallization from aq. 30% ethanol, gave (–)-α-*hexyl-γ-carboxymethyltetronic acid*, needles, m.p. 205° (decomp.), $[\alpha]_{5461}^{20}$ – 74° in ethanol (*c*, 1), light-absorption values in ethanol $\lambda_{max.}$ 230 mμ, log ε 4·08 (Found: C, 59·4; H, 7·6; equivalent 121. $C_{12}H_{18}O_5$ requires C, 59·5; H, 7·85%; mol.wt. 242).

Degradative studies on viridicatic acid

Acid hydrolysis. Viridicatic acid (1·035 g.) was hydrolysed under reflux with boiling 2 N-H_2SO_4 (60 ml.) in a stream of CO_2-free N_2, the effluent gases passing successively through bubblers containing Brady's reagent and standardized aq. $Ba(OH)_2$, which was titrated at intervals. The CO_2 liberated after 3, 5, 10, 15 and 25 hr. corresponded with 78·5, 105·5, 115·0, 128·5 and 147·0 ml. of 0·1 N-$Ba(OH)_2$ respectively. The amount required for liberation of 2 mol. of CO_2/mol. of viridicatic acid is 161·7 ml. of 0·1 N-$Ba(OH)_2$. One molecule of CO_2 was therefore eliminated rapidly, the second molecule much more slowly. There was a small amount of precipitate in the bubbler with Brady's reagent. The hydrolysis mixture was neutralized with N-NaOH and evaporated under reduced pressure to dryness several times, water being added after each distillation. The combined distillates made up to 500 ml. were investigated as follows: the distillate (i) reduced Fehling's solution in the cold, (ii) gave a positive Voges–Proskauer reaction (indicating the probable presence of acetoin or diacetyl) and (iii) gave slowly with Brady's reagent the bisdinitrophenylhydrazone of diacetyl, m.p. 321° after recrystallization from a mixture of toluene and nitrobenzene (Found: C, 43·3; H, 3·5; N, 25·1. $C_{14}H_{14}O_8N_8$ requires C, 43·1; H, 3·2; N, 25·1%). The m.p. was not depressed on admixture with an authentic specimen. The amount of the bisdinitrophenylhydrazone of diacetyl obtained from the total distillate was 1·627 g., whereas the theoretical amount, assuming 1 mol. of acetoin from 1 mol. of viridicatic acid, is 1·776 g.

An attempt to isolate the volatile acid after this prolonged hydrolysis was unsuccessful; it may have been slowly removed despite the reflux condenser.

Isolation of n-hexanoic acid. In another experiment viridicatic acid (1·02 g.) was hydrolysed with 100 ml. of N-H_2SO_4 for 4·5 hr. The hydrolysate was then distilled and the distillate collected. Distillation was repeated after addition of water to the residue until no more volatile acid was obtained. The total distillate required 33·7 ml. of 0·1 N-NaOH for neutralization to phenolphthalein, corresponding with 85% of 1 mol. of volatile acid from 1 mol. of viridicatic acid.

(i) Isolation as *p*-bromophenacyl ester. The sodium salt in aqueous ethanol was refluxed with the theoretical amount of *p*-bromophenacyl bromide under the usual conditions and afforded the *p*-bromophenacyl ester of *n*-hexanoic acid as platelets of m.p. 72°, not depressed on admixture with the ester derived from authentic *n*-hexanoic acid (Found: C, 53·95; H, 5·6; Br, 25·4. Calc. for $C_{14}H_{18}O_3Br$: C, 53·7; H, 5·4; Br, 25·6%). Thus the fatty acid formed as hydrolytic product was identified as *n*-hexanoic acid.

(ii) Isolated as amide. The acid (0·21 g.) was extracted with ether from 100 ml. of the volatile acid distillate and converted into the methyl ester with diazomethane. The ester was treated with 10 ml. of conc. aq. NH_3 soln. and kept at 0° for 2 days. It was then neutralized with 2 N-HCl and extracted with ether, which afforded colourless needles (0·23 g.) of m.p. 99–100°, recrystallized from light petroleum to a constant m.p. of 101°. The m.p. was not depressed by admixture with an authentic sample of *n*-hexanoamide [Found: C, 62·7; H, 11·0; N, 12·2. $C_6H_{13}ON$ requires C, 62·55; H, 11·4; N, 12·2%].

Isolation of β-hydroxylaevulic acid as dinitrophenylhydrazone. Viridicatic acid (0·41 g.) was hydrolysed with 2 N-H_2SO_4 (25 ml.) for 4 hr. and the product was evaporated to small volume under reduced pressure. The residue was

extracted with ether and the oily material so obtained was dissolved in water and treated with Brady's reagent. The mixture was kept overnight, then the crude dinitrophenylhydrazone was collected and purified by dissolving in aq. $NaHCO_3$ and reprecipitating. It was finally recrystallized from aq. ethanol and afforded orange needles of the (−)-2:4-*dinitrophenylhydrazone* of β-hydroxylaevulic acid, m.p. 163° (decomp.), $[\alpha]_{5461}^{20}$ − 63° in ethanol; (*c*, 0·2) [Found: C, 42·45; H, 4·0; N, 18·0. Calc. for $C_{11}H_{12}O_7N_4$ (from $C_5H_8O_4$): C, 42·3; H, 3·9; N, 17·95%]. It gave no depression in m.p. when mixed with an optically inactive synthetic sample prepared for comparison.

Reaction with bromine: preparation of (−)-α-bromo-γ-carboxymethyltetronic acid. (*a*) Viridicatic acid (0·489 g.) was dissolved in acetic acid (25 ml.) and treated dropwise with 1·3 N-bromine in acetic acid. There was no decolorization. After 0·5 ml. of the bromine solution had been added the mixture was kept for 1 hr. and then evaporated at room temperature over KOH in high vacuum. The product (0·49 g.), m.p. 174–175°, proved to be unchanged viridicatic acid. No bromination occurred therefore under these conditions.

(*b*) Viridicatic acid (0·480 g.) was dissolved in aq. 50% (v/v) acetic acid (25 ml.) and 1·3 N-bromine in the same solvent was added. Immediate decolorization occurred and a sharp end-point was obtained after the equivalent of 2 mol. of bromine had been added. The solution was rapidly evaporated at 30° over KOH and yielded a yellowish-brown crystalline mass (0·495 g.). This was treated with 8 ml. of $CHCl_3$ and filtered. The solid thus obtained was washed with ether–light petroleum (1:1) and afforded a colourless solid of m.p. 193–194°, recrystallized to give (−)-α-bromo-γ-carboxymethyltetronic acid (0·26 g.) of m.p. 194° (decomp.). The acid gave a red with aq. $FeCl_3$ and a violet with $NaNO_2$ [Found: C, 30·1; H, 2·05; Br, 33·7; equivalent (phenolphthalein), 119. Calc. for $C_6H_5O_5Br$: C, 30·4; H, 2·1; Br, 33·7%; mol.wt. 237]. The product had $[\alpha]_{5461}^{20}$ − 120° in water (*c*, 0·56), showed no depression in m.p. when mixed with authentic material prepared similarly from carlosic acid, for which $[\alpha]_{5461}^{20}$ − 117° in water (*c*, 0·562) is reported (Clutterbuck *et al.* 1935).

Preparation of synthetic (±)-β-hydroxylaevulic acid 2:4-dinitrophenylhydrazone. β-Bromolaevulic acid (1·05 g.), prepared according to Overend, Turton & Wiggins (1950), was hydrolysed in 0·5 N-KOH (20 ml.). After half an hour at room temp. an excess of Brady's reagent was added. The crude dinitrophenylhydrazone was collected after 1 hr. and purified by dissolution in and reprecipitation from aq. $NaHCO_3$. The orange–yellow solid (0·58 g.; m.p. 158–160°) was recrystallized from aq. ethanol (50%, v/v) to yield the *(±)-β-hydroxylaevulic acid 2:4-dinitrophenylhydrazone* of m.p. 163° (decomp.) [Found: C, 42·6; H, 4·0; N, 17·6. $C_{11}H_{12}O_7N_4$ requires C, 42·3; H, 3·9; N, 17·95%].

SUMMARY

1. From the culture filtrate of a strain of *Penicillium viridicatum* Westling, G.A. 834, two crystalline acids were obtained, namely terrestric acid and a new acid, viridicatic acid.

2. Viridicatic acid, $C_{12}H_{16}O_6$, m.p. 174·5°, $[\alpha]_{5461}^{20}$ − 105° in ethanol (*c*, 1), titrates as a dibasic acid, and has properties close to those of the tetronic acid derivative carlosic acid.

3. Derivatives of viridicatic acid prepared were the mono-*p*-bromophenacyl ester, m.p. 188·5°, and semicarbazone, m.p. 198–199°. Catalytic hydrogenation afforded (−)-α-hexyl-γ-carboxymethyltetronic acid, m.p. 205° (decomp.), and bromine in aqueous acetic acid gave (−)-α-bromo-γ-carboxymethyltetronic acid.

4. On short acid hydrolysis viridicatic acid affords carbon dioxide, *n*-hexanoic acid and β-hydroxylaevulic acid; on prolonged hydrolysis a further molecule of carbon dioxide and of acetoin are obtained by degradation of the hydroxylaevulic acid.

5. These facts and other properties such as light-absorption values establish the structure of viridicatic acid as (−)-α-hexanoyl-γ-carboxymethyltetronic acid. The relationship between viridicatic acid and carlosic acid is thus similar to that between terrestric and carolic acid, since the first member of each pair differs from the second only by the presence of an additional ethyl group.

6. The mycelium of strain G.A. 834 afforded mannitol, *i*-erythritol, ergosteryl palmitate and viridicatin.

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