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STRUCTURE OF CITREOVIRIDIN, A MYCOTOXIN PRODUCED BY *PENICILLIUM CITREO-VIRIDE* MOLDED ON RICE[1]

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Abstract—Structure of citreoviridin, a "yellowed rice toxin" produced by *P. citreo-viride* molded on rice, was determined as **1b**.

The so-called "yellowed rice" is rice on which a fungus is parasitic and produces toxic yellow substances. There have been found three kinds of yellowed rice: the toxicarium-, islandia- and citrinum-yellowed rice, which are rice moulds produced by *P. toxicarium* Miyake[2] (or *P. citreo-viride* Biourge), *P. islandicum* Sopp, and *P. citrinum* Thom, respectively. The latter two were found after the World War II, whereas the former was first recognized in Formosan rice in 1937[2]. Since 1940 a series of studies on the toxicity of the toxicarium-yellowed rice have been carried out:[3] toxic symptoms being nerve paralysis similar to beri-beri. Hirata isolated a yellow compound named citreoviridin from the mouldy rice and studied its chemical properties[4] [LD_{50} 7.2 mg/Kg mouse (i.p.); 29 mg/Kg mouse (orally)].[3] Later, *P. ochrosalmoneum* Udagawa[5] and *P. pulvillorum* Turfitt[6] were found to produce the same compound.

The structure of the toxic substances produced by *P. islandicum* has been extensively studied by Shibata *et al.* who isolated many bis-anthraquinone derivatives such as luteoskyrin[7] (LD_{50} 1.47 mg/10 g mouse), and a polyene named erythroskyrin.[8] *P. islandicum* also produces a chlorine-containing toxic peptide, islanditoxin[9] (= cycloclorotine?[9a]) (LD_{50} 4.75 μg/10 g mouse). These toxic compounds cause liver injuries.[3] The citrinum-yellowed rice contains citrinin, a toxic component,[10] which injures kidneys.

Citreoviridin isolated from *P. citreo-viride* cultured on rice, crystallizes from methanol in orange-yellow needles with the molecular formula $C_{23}H_{30}O_6 \cdot CH_3OH$, from which the methanol of crystallization can be eliminated by dissolving in chloroform and drying in a desiccator. On acetylation it forms a monoacetate (**2**), and on *p*-nitrobenzoylation, a mono-(**3**) ($\nu_{max}^{CHCl_3}$ 3560 cm^{-1}) and a di-*p*-nitrobenzoate (**4**) (no OH band in IR), indicating the presence of two OH groups in **1**.

Permanganate oxidation of **1** in pyridine afforded a carboxylic acid (**5**). This acid has the molecular formula $C_8H_8O_4$ and exhibits UV absorption max at 293 nm (ε 6400) and an acidic pK_a' at 2.8. The NMR spectrum of its methyl ester (**6**) shows the presence of one Me group (2.25 ppm, s), two OMe groups, one of which is attributable to the methoxycarbonyl group introduced by methylation, and one vinyl proton (5.78, s). The IR bands at 1720, 1630 and 1558 cm^{-1} and the UV max at 294 nm (ε 6030) of the methyl ester (**6**) suggest that **4** is an α-pyrone, and not a γ-pyrine, derivative.[11] Thus, the acid (**5**) can be represented by partial formula **5a**. That the 6-position in **5a** has a substituent is evident from the signal of the vinyl proton at 5.78 ppm, since singals of C-6 proton in α-pyrone derivatives are usually around 7.5 ppm.[12] When a limited amount of permanganate was used at a low temp., the corresponding aldehyde (**7**) was obtained.

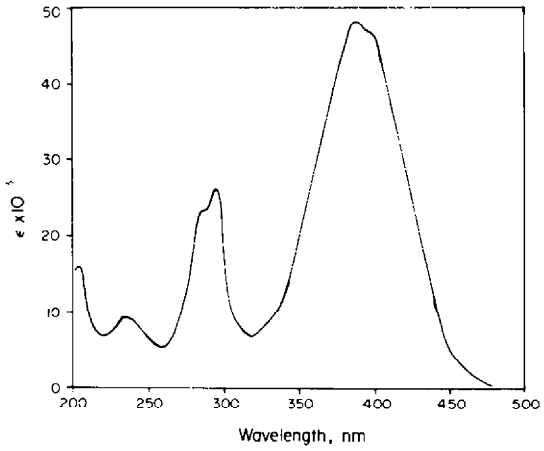
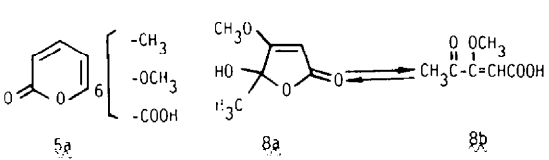


Fig. 1. UV spectrum of citreoviridin in methanol.

Ozonolysis of **1** gave glyoxal and diacetyl, both of which were identified as their 2,4-dinitrophenylhydrazone. The *p*-nitrobenzoate **3**, on ozonization followed by chromatography gave an oily substance (**8**), which gave a diacetyl 2,4-dinitrophenylhydrazone. NMR signals of **8** [1.65 ppm (3H, s), 3.98 (3H, s), 5.10 (1H, s), and 5.65 (1H, br)] coupled with its IR bands (3340, 1746, 1643 cm^{-1}) suggest a γ-lactone structure (**8a**) which may be in equilibrium with the acid (**8b**). Diacetyl could be produced from **8a** by acid hydrolysis followed by decarboxylation.



Formula 1

Partial catalytic hydrogenation (3.06 moles of H_2 was absorbed) of **1** gave an oily mixture, the UV [λ_{max}^{MeOH} 287 (ε 5600)] and IR spectra ($\nu_{max}^{CHCl_3}$ 1695, 1615 cm^{-1}) of

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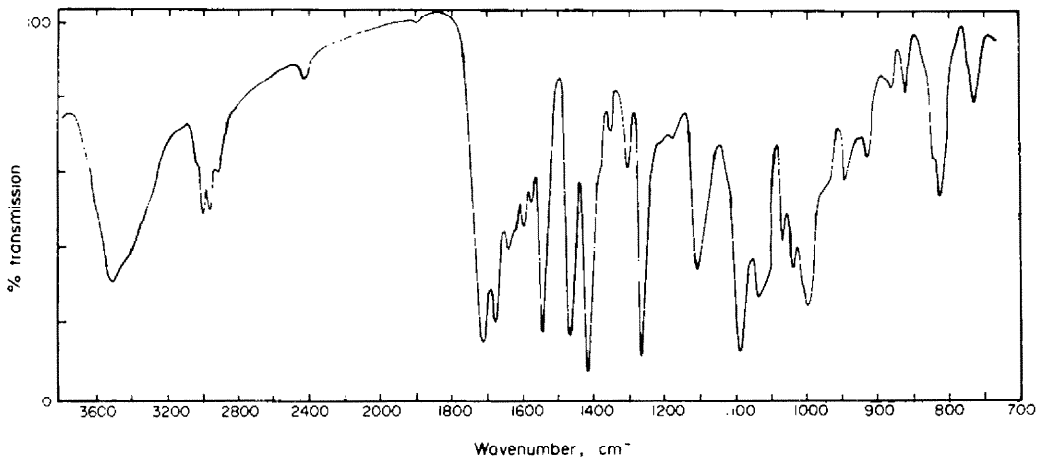
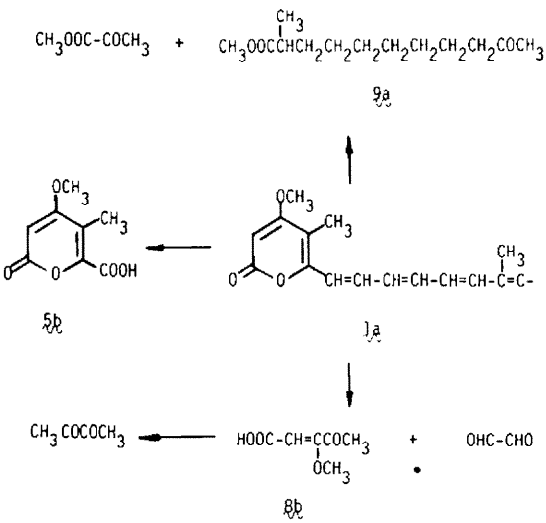


Fig. 2. IR spectrum of citreoviridin (KBr disc).

which indicate partial remains of the pyrone moiety. Ozonization followed by steam-distillation of the product gave methyl pyruvate which was identified as its 2,4-dinitrophenylhydrazone. The residue of the steam-distillation, on methylation with diazomethane, afforded an oily methyl ester (**9**), which has a molecular weight 228 as determined by mass spectrometry. It showed a positive iodoform test, and gave a 2,4-dinitrophenylhydrazone. The NMR spectrum of **9** shows the following signals: 0.96 ppm (3H,d, sec-Me), 1.38 (10-12H,br, sat. C-H), 2.30 (3H, s, CH_3CO), 2.50 (3H,br,-COCH) and 4.50 (3H,s, CH_3O). The structure of **9** must be represented by **9a**, in which the methoxycarbonyl and the Me groups are vicinal to each other since they must have been present in the pyrone moiety of **1**.



Formula 2

The above results indicate the partial formula **1a** for citreoviridin. The UV maximum of **1** at 388 nm (ϵ 48,000) suggests that the pyrone chromophore is in conjugation with a polyene moiety, and the NMR spectrum of **1** (Fig. 3) also supports the partial formula **1a**.

Citreoviridin di-*p*-nitrobenzoate (**4**), when ozonized, gave a saturated aldehyde (**10**). The NMR spectrum of **10** indicates the presence of the groups shown below.

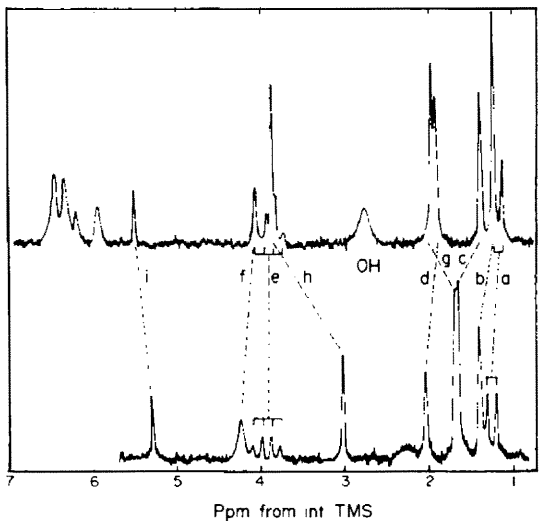
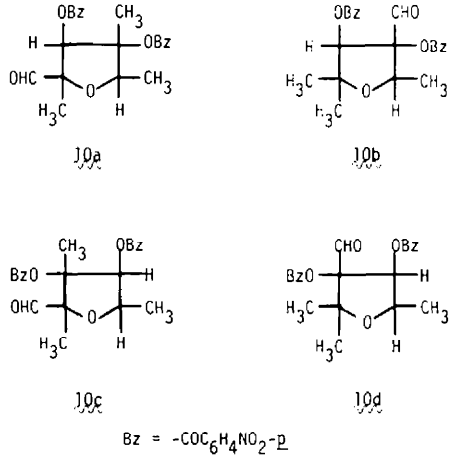


Fig. 3. NMR spectra of citreoviridin at 60 MHz. Upper spectrum: in chloroform-d; lower spectrum: in benzene.

	Group	NMR signal (in ppm)
(a)	$CH_3-C^*(-O-)-$	1.29, singlet, 3H
(b)	$CH_3-C^*(-O-)-$	1.70, singlet, 3H
(c)	$CH_3-CH(-O-C^*\dagger)-$	1.53, doublet, J = 6.5 Hz, 3H
		4.46, quartet, J = 6.5 Hz, 1H
(d)	$H-C\dagger(-O-Bz)-$	6.46, singlet, 1H
		8.36, singlet, 4H
(e)	$-C^*(-O-Bz)-$	8.06, 8.21, 8.29, 8.44, $A_2'B_2'$ type quartet, 4H
(f)	$-C^*(-CHO)-$	9.60, singlet, 1H

Bz = *p*-nitrobenzoyl; the C atoms bearing the same mark, (*) or (†), may be the same C atom.

All of the corresponding signals, except that of the aldehydic proton, are present in the NMR spectrum of the di-*p*-nitrobenzoate (**4**). Since on *p*-nitrobenzoylation of **1** the corresponding quartet to that in group (c) was not much shifted (0.21 ppm in **3** and 0.46 ppm in **4**) as expected if CH_3CH–OH were transformed to CH_3CH–$OCOC_6H_4NO_2(p)$, the O atom in (c) must link with two C atoms. To accommodate the above groups into the molecular formula, only the following four formulas, **10a** to **10d**, are possible.

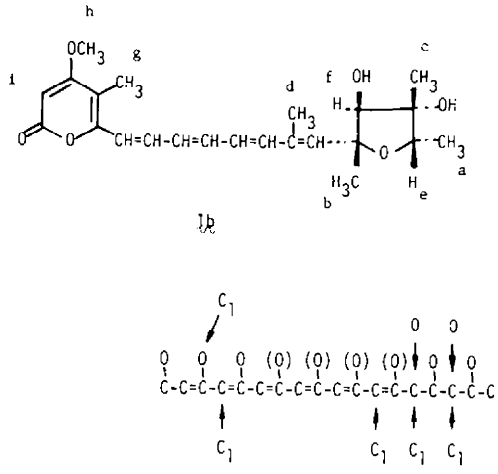


Formula 3

Since the quartet in (c) and the singlet in (d) show no indication to couple mutually and since the same is true in the NMR of the lactone **12** (*vide infra*) whose conformation is drastically changed from that of **10**, two protons on the tetrahydrofuran nucleus must not be in a vicinal position. Thus, the formulas **10c** and **10d** can be excluded.

The aldehyde **10** was oxidized to the corresponding acid (**11**). Hydrolysis of this acid with base, followed by acidification, afforded an oily 5-membered lactone (**12**) as indicated by its IR band at 1798 cm^{-1}. The aldehyde, therefore, must have the structure **10a**, since **10b** cannot produce a 5-membered lactone. Relative configuration of **10** is deduced as **10e** from the following evidence: (a) the 4-OH is *cis* to the 2-aldehyde since the corresponding acid (**11**) forms a γ-lactone; (b) two vicinal OH groups are *trans* to each other since **1** consumes periodate only very slowly; and (c) the C-5 proton is *trans* to C-3 proton as deduced from the fact that the *p*-nitrobenzoyl group at 4-position affects the NMR signal of the C-3 proton very strongly (Δ 1.31 ppm), whereas only a small shift (Δ 0.26 ppm) is observed for the C-5 proton in comparison with the NMR spectra of **3** and **4**.

The structure of **1** is now represented by the formula **1b**, which is reasonable for the biogenetic point of view.



Formula 5.

It may be worth noting that some of the NMR signals of **1** are strongly shifted by change of the solvent from $CDCl_3$ to C_6H_6 as shown in Fig. 3. The signals which move to higher fields by the solvent change belong to the pyrone moiety, whereas slight down-field shifts are observed on the signals belonging to the tetrahydrofuran moiety. The pyrone nucleus is planar and forms a π complex with benzene, anisotropy of which may shift the signals of the pyrone group to higher fields.

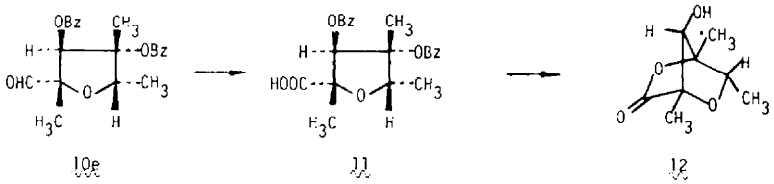
The structure and the relative configuration of citreoviridin (**1b**) were later confirmed by X-ray analysis,[13] which also clarified the polyene moiety as having an all-*trans* configuration.

Biosynthetic studies by Nagel *et al.*[14] indicated that **1** is indeed produced from 9 molecules of acetic acid and 5 molecules of methionine (C_1-unit) as expected.

EXPERIMENTAL

All m.ps are uncorrected. the spectra were recorded on the following instruments: IR spectra, JASCO DS-402G and IR-S (s, strong; m, medium; w, weak); UV spectra, Beckman DK-2; NMR spectra, JEOL JNM-3 (60 MHz); chemical shifts (δ) are represented in ppm from int TMS and solvent is $CDCl_3$ unless otherwise indicated; mass spectra, Hitachi RMU-6D spectrometer. Unless otherwise noted the analytical samples were dried over P_2O_5 at 60° for 6–12 hr *in vacuo*. Abbreviation: 2,4-DNP = 2,4-dinitrophenylhydrazone.

Citreoviridin (**1**). Washed rice (400 ml) in an Erlenmeyer flask (1 l) was pasteurized (100°, 20 min, ×3), then planted with *P. citreo-viride*, and incubated at 20–24° for 30–40 days. The flask was shaken well everyday. During the period of time, the rice turned yellow owing to pigments produced. It was extracted with acetone and the extract was evaporated *in vacuo*, when a yellow solid precipitated. The solid was again dissolved in acetone and



Formula 4.

reprecipitated by the addition of petroleum ether. The ppts were dissolved in a small quantity of benzene and chromatographed on acid-washed alumina. Methanol-ether (1:10) eluates contained citreoviridin which was then crystallized from MeOH or pyridine-water.

Citreoviridin (**1**) crystallized from MeOH: orange-yellow needles; m.p. 107–111°; $[\alpha]_D$ −68.9°; UV in Fig. 1; IR in Fig. 2; δ 1.17 (3H,d, J=7 Hz), 1.23 (3H,s), 1.40 (3H,s), 1.91 (3H,s), 1.95 (3H,s), 2.75 (2H,br,OH), 3.84 (1H,q,J=7 Hz), 3.85 (3H,s), 4.07 (1H,s), 5.52 (1H,s), 5.96 (1H,s), 6.34 and 6.45 (6H,m); Kuhn–Roth C-CH_3 determination gave 5 C-CH_3 groups and catalytic hydrogenation gave *ca.* 7 double bonds. [Found: C, 66.88, 66.27; H, 8.01, 7.81; OCH_3 13.92%; mol. wt. 446 (Rast). $C_{23}H_{30}O_6 \cdot CH_3OH$ requires: C, 66.34; H, 7.89; OCH_3 (2) 14.3%; mol. wt. 434.5].

Citreoviridin (**1**) crystallized from aqueous pyridine: yellow needles; m.p. 117–121°. [Found: C, 65.80; H, 7.56; OCH_3 7.37. $C_{23}H_{30}O_6 \cdot H_2O$ requires: C, 65.69; H, 7.67; OCH_3 (1) 7.38%].

Citreoviridin monoacetate (**2**). A solution of citreoviridin (100 mg) in a mixture of pyridine (0.06 ml) and Ac_2O (2 ml) was heated to 50° for 5 hr. Addition of water to the mixture caused precipitation of a solid, which was crystallized from 80% aq. MeOH to give yellow needles, m.p. 99–101°; λ_{max}^{MeOH} 388 nm (ϵ 49,400), 293 (29,400), 285sh (26,600), 234 (14,800), 204 (19,800); $\nu_{max}^{CHCl_3}$ 3550w, 1740s, 1700s, 1627m, 1535m, 1455s, 1407s, 1374m, 1245s, 1140m, 1095m, 1043m, 998m, 810m cm^{-1}; δ 1.13 (3H,s), 1.18 (3H,d,J=7Hz), 1.28 (3H,s), 1.93 (6H,s), 2.15 (3H,s), 2.20 (1H,br,OH), 3.79 (3H,s), 3.83 (1H,q, J = 7Hz), 5.03 (1H,s), 5.44 (1H,s), 5.84 (1H,s,br), 6.34 and 6.44 (6H,br). [Found: C, 62.83; H, 7.54; OCH_3 6.01; CH_3CO 9.85%. $C_{25}H_{32}O_7 \cdot 2H_2O$ requires: C, 62.48; H, 7.55; OCH_3(1) 6.45; CH_3CO (1) 8.95%].

The solid, when crystallized from methanol, gave an anhydrous monoacetate (**2**), m.p. 109–111.5°. [Found: C, 67.02; 67.24, 66.92; H, 7.30, 7.68, 7.37; OCH_3 6.58. $C_{25}H_{32}O_7$ requires: C, 67.55; H, 7.26; CH_3O (1) 7.00%].

Hydrolysis of **2** with $Ba(OH)_2$ in aq. MeOH at 60° for 5 min gave **1**, which was identified by NMR spectra.

Citreoviridin mono-p-nitrobenzoate (**3**) *and di-p-nitrobenzoate* (**4**). To a solution of **1** (2.2 g) in dry pyridine (7 ml) was added *p*-nitrobenzoyl chloride (3.7 g) and the mixture was allowed to stand at room temp. overnight. Addition of water (20 ml) to the mixture gave a ppt which was collected and extracted with MeOH. The MeOH-soluble fraction was crystallized several times from MeOH to give **3** (1.5 g), m.p. 178–178.5°; λ_{max}^{MeOH} 405 nm (ϵ 50,000), 387 (53,4000), 295 (31,100), 285 (28,600); $\nu_{max}^{CHCl_3}$ 3560w, 1728s, 1695s, 1535s, 1455s, 1408s, 1348m, 1272s, 1249s, 1100s, 1000s; δ 1.20 (3H,s), 1.26 (3H,d, J = 6.5 Hz), 1.39 (3H,s), 1.94 (3H,s), 2.00 (2H,s), 2.35 (1H,br, OH), 3.85 (3H,s), 4.05 (1H,q, J = 6.5 Hz), 5.10 (2H,s), 5.95 (1H,s), 6.40 and 6.46 (6H,m), 8.25 (4H,s). (Found: C, 65.04, 65.01; H, 6.32, 6.15; N, 2.42, 2.68, $C_{30}H_{33}O_9N$ requires: C, 65.32; H, 6.03; N, 2.54%).

The MeOH-insoluble residue was crystallized from pyridine to give **4** (0.94 g), m.p. 269–271°; λ_{max} 405 nm (ϵ 50,300), 387 (54,100), 295 (29,900), 285 (31,200), 265 (36,300); $\nu_{max}^{CHCl_3}$ 1730s, 1695s, 1630w, 1610w, 1535s, 1455m, 1410s, 1350m, 1270s, 1250s, 1100s, 1015m, 1000s; δ 1.37 (3H,s), 1.52 (3H,s, J = 6.5 Hz), 1.67 (3H,s), 1.92 (3H,s), 1.96 (3H,s), 3.84 (3H,s), 4.30 (1H,q; J = 6.5 Hz), 5.46 (1H,s), 5.86 and 6.12 (6H,m), 6.41 (1H,s), 8.14 and 8.16 (central two peaks of $A_2'B_2'$), 8.31 (4H,s). (Found: C, 63.48, 63.53; H, 5.55, 5.27; N, 4.07, 4.12. $C_{37}H_{36}O_{12}N_2$ requires: C, 63.50; H, 5.15; N, 4.00%).

4-Methoxy-5-methyl-α-pyrone-6-carboxylic acid (**5**). To a solution of **1** (1.0 g) in pyridine (14 ml) and water (57 ml) was added at 0° with stirring pulverized $KMnO_4$ (2.57 g). The mixture was adjusted to pH 4–5 with dil H_2SO_4 and treated with $NaHSO_3$ to dissolve MnO_2. The solution was then acidified to *ca.* pH 2 with dil H_2SO_4, and extracted with ether in a continuous extraction apparatus for 10 hr. From the ether layer precipitated crystals, which were recrystallized from MeOH to give colorless needles, m.p. 216°; pK_a' (in H_2O) 2.8; λ_{max}^{MeOH} 293 nm (ϵ 6400); ν_{max}^{KBr} 3450m, 2900–2600w, 1720s, 1653s, 1560m, 1388m, 1280m. [Found: C, 51.75; H, 4.45%; mol. wt. 182 (titration). $C_8H_8O_5$ requires: C, 52.18; H, 4.38%; mol. wt. 184.14].

Treatment of **5** with diazomethane in ether gave **6**, m.p. 131–132°; λ_{max}^{MeOH} 294 nm (ϵ 6030); ν_{max}^{KBr} 1734s, 1723s, 1710w, 1661w, 1565m, 1451m, 1436m, 1398m, 1311s, 1265s, 1145m, 1076s, 995w, 930m, 884m, 857m cm^{-1}; δ 2.26 (3H,s); 3.91 (6H,s), 5.80 (1H,s).

4-Methoxy-5-methyl-α-pyrone-6-aldehyde (**7**). To a solution of **1** (1.0 g) in pyridine (20 ml) and water (15 ml) was added dropwise at −10° a solution of $KMnO_4$ (0.62 g) in water (20 ml). The mixture was worked up in a same manner as described in the preparation of **5**. After continuous extraction with ether for 72 hr, the water layer was extracted with EtOAc for a further 72 hr. The EtOAc extract was evaporated and the residue was chromatographed on a silica gel column using $CHCl_3$ as eluant to afford **5** which was crystallized from MeOH, m.p. 140–141°; λ_{max}^{MeOH} 284 nm (ϵ 6700). (Found: C, 54.01, 53.62; H, 6.04, 6.01. $C_8H_8O_4 \cdot CH_3OH$ requires: C, 53.99; H, 6.04%).

Ozonolysis of citreoviridin (**1**). A solution of **1** (500 mg) in $CHCl_3$ (50 ml) was ozonized at −5° for 15 hr. The mixture was distilled with water (10 ml) in the presence of Pd–C (10%, 20 mg) under N_2, and the distillate was trapped successively with an empty trap, a trap containing acidified 2,4-dinitrophenylhydrazine solution (50 ml), an empty trap, and a trap containing aq. $Ba(OH)_2$. The first empty trap was washed with the hydrazine solution and the red ppt was triturated with benzene. From the benzene-soluble fraction was obtained formaldehyde 2,4-DNP (10 mg), which was identified by mixed m.p. determination and comparison of UV and IR spectra with those of an authentic sample. The benzene-insoluble fraction was crystallized from nitrobenzene to give glyoxal 2,4-DNP (50 mg) which was identified by comparison of UV and IR spectra with those of an authentic sample. The second trap gave a red ppt, which was washed with benzene to give diacetyl 2,4-DNP, m.p. 280–300°, identified with an authentic sample by comparison of UV and IR spectra. (Found: C, 43.72; H, 3.05. Calc. for $C_{16}H_{14}O_8N_8$: C, 43.05; H, 3.16%). The last trap gave $BaCO_3$ (60 mg).

Ozonolysis of citreoviridin mono-p-nitrobenzoate (**3**). The benzoate **3** (1.13 g) was ozonized in CH_2Cl_2 (100 ml) at −70° for 3 hr. After removal of the solvent *in vacuo*, the ozonide was dissolved in MeOH (40 ml) and decomposed with Pd–C (10%, 500 mg) in H_2 atm. (*ca.* 4 moles of H_2 absorbed) to give an oily material, which was chromatographed on a silica gel column. Elution with $CHCl_3$ afforded **8** as an oil, which shows the following NMR and IR spectra: δ 1.65 (3H,s), 3.98 (3H,s), 5.10 (1H,s), 5.65 (1H,s,br); ν_{max}^{film} 3350br, 1745sbr, 1640s, 1380m, 1345m, 1275m, 1230s, 1200s, 1130m, 1082m, 980m, 895m, 810m, 785m cm^{-1}.

Treatment of **8** with 2,4-dinitrophenylhydrazine solution afforded diacetyl 2,4-DNP as identified by IR. (Found: C, 43.92, 43.92; H, 3.54, 3.58; N, 24.85, 24.87. Calc. for $C_{16}H_{14}O_8N_8$: C, 43.05; H, 3.16; N, 25.11%).

Methyl 2-methyl-10-ketoundecylate (**9**) *and methyl pyruvate from hydrogenated* **1**. A solution of **1** (0.20 g) in MeOH (20 ml) was hydrogenated in the presence of PtO_2 (10 mg) for 2 hr. After 3.06 moles of H_2 was absorbed, the mixture was filtered and concentrated *in vacuo*. The residue was chromatographed on a silica gel column using benzene as eluant to give an oily product; λ_{max}^{MeOH} 287 nm (ϵ 5600), 230 (5000), 208 (17400); $\nu_{max}^{CHCl_3}$ 1695, 1615 cm^{-1}. The oily product (150 mg) was dissolved in $CHCl_3$ (50 ml) and the solution was ozonized at 0° for 24 hr. The ozonide solution was mixed with 30% H_2O_2 (2 ml) and water (10 ml), and the mixture, after heating under reflux for 1 hr, was steam-distilled. Treatment of the distillate with 2,4-dinitrophenylhydrazine solution afforded methyl pyruvate 2,4-DNP, m.p. 152–152.5°, which was identified by mixed m.p. determination, and UV and IR spectral comparisons with an authentic sample. (Found: C, 43.22, 42.90; H, 3.88, 3.83; N, 20.43, 20.36. Calc. for $C_{10}H_{10}O_6N_4$: C, 42.56; H, 3.57; N, 19.85%).

The residue of the steam-distillation was acidified with HCl to pH 2 and extracted with ether. The extract was evaporated to dryness and the residue was treated with diazomethane in ether. The methylated product was purified by GLC (Apieson M 2m column at 170°; carrier gas He) to give **9** as a homogeneous oil; positive iodoform test; MS *m/e* 228 (M^+), 197, 171; ν_{max}^{film} 1740s, 1720s; δ 0.96 (3H,d, J = 7 Hz), 1.38 (10–12H,m), 2.30 (3H,s), 2.50 (3H,m), 4.05 (3H,s).

The oil **9** (20 mg) in MeOH (0.5 ml) was treated with 2,4-dinitrophenylhydrazine solution to give a ppt which was chromatographed on alumina (elution with benzene). The yellow eluates contained the 2,4-DNP derivative of **9**, m.p. 82–

82.5°; ν_{max} 3450, 1735, 1620, 1590 cm^{-1}. (Found: C, 55.81; H, 7.09. $C_{19}H_{28}O_6N_4$ requires: C, 55.87; H, 6.91%).

Periodate oxidation of citreoviridin (**1**). Into a mixture of 2N H_2SO_4 (2 ml), 0.1N HIO_4 (8 ml), and MeOH (30 ml) was added **1** (22 mg). The residual periodate was determined iodometrically. No periodate consumption was observed after 11 hr at room temp., but after 100 hr 0.8 mole of periodate was consumed.

The aldehyde **10**. Ozonization of the di-*p*-nitrobenzoate **4** in CH_2Cl_2 followed by decomposition with water gave a white solid (**10**), which was purified by silica gel chromatography (elution with $CHCl_3$) and crystallization from MeOH, m.p. 205–208°; iodoform test negative; $\nu_{max}^{CHCl_3}$ 1740s, 1610m, 1530s, 1387m, 1352s, 1322m, 1270s, 1117s, 1105s, 1015m, 875m, 840m cm^{-1}; NMR see text. (Found: C, 55.45; H, 4.33; N, 5.87. $C_{22}H_{20}O_{10}N_2$ requires: C, 55.93; H, 4.27; N, 5.93%)

The acid **11**. To a solution of **10** in aq. pyridine was added with stirring $KMnO_4$ solution. After 10 hr under stirring, the excess $KMnO_4$ was decomposed by addition of $NaHSO_3$ and the mixture was filtered. Acidification of the filtrate gave a ppt, which crystallized from $CHCl_3$-pet. ether to give **11**, m.p. 230–233°; $\nu_{max}^{CHCl_3}$ 1775w, 1745s, 1610m, 1530s, 1352s, 1265s, 1100s, 1017m, 875m, 835m cm^{-1}. (Found: C, 53.83; H, 4.48. $C_{22}H_{20}O_{11}N_2$ requires: C, 54.10; H, 4.13%).

The lactone **12**. A solution of **11** (100 mg) in 2% KOH in 90% MeOH (2 ml) was boiled for 3 hr. After evaporation of the solvent, the mixture was acidified with 2N HCl, when *p*-nitrobenzoic acid precipitated. The ppt was filtered off and the filtrate was evaporated. The residue was extracted with $CHCl_3$ and chromatographed on a silica gel column to give **12** (26 mg) as an oil; iodoform test negative; ν_{max}^{film} 3430 br, 1800s, 1500m, 1390m, 1276m, 1180s, 1110s, 1086s, 1010s, 920s, 880m, 845m cm^{-1}.

The lactone **12** was treated in pyridine with 3,5-dinitrobenzoyl chloride and the product was chromatographed on a silica gel column. Elution with $CHCl_3$ gave a solid which was crystallized from benzene-pet. ether to give the lactone 3,5-dinitrobenzoate, m.p. 75–87°; $\nu_{max}^{CHCl_3}$ 3100m, 1815s, 1750s, 1632m, 1550s, 1455m, 1390m, 1345s, 1265s, 1160s, 1087s, 1046m, 1012m, 921s, 880m; δ 1.33 (3H,d J = 6Hz), 1.48 (3H,s), 1.57 (3H,s), 4.52 (1H,q, J = 6Hz), 7.34 (s, benzene), 9.23 (2H,d, J = 2 Hz), 9.34 (1H,d, J = 2 Hz). [Dried at 45° *in vacuo*: Found: C, 55.07, 54.90; H, 4.53, 4.53; N, 6.78. $C_{15}H_{14}O_9N_2 \cdot 0.8\ C_6H_6$ (partial loss of benzene for crystallization) requires: C, 55.23; H, 4.40; N, 6.57%].

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