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THE STRUCTURE OF CITREOVIRIDIN, A TOXIC COMPOUND PRODUCED BY P. CITREOVIRIDE MOLDED ON RICE

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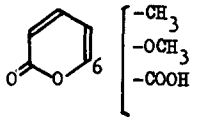
In 1937, P. toxicarium Miyake was found in Formosan rice by Miyake (1). Since 1940 a series of studies on the toxicity of the fungus molded on rice have been extensively carried out by many workers (2), and one of us (Y.H.) isolated from the rice a toxic yellow compound which was named citreoviridin, since later P. citreoviride was found to produce the same substance (3). Tsunoda found that P. ochrosalmoneum Udagawa, which was found by Udagawa in 1959 (4), also produced citreoviridin when being parasitized on rice (5).

Citreoviridin (I), when being crystallized from methanol, forms yellow crystals, m.p. 107-111°, having a molecular formula $C_{23}H_{30}O_6 \cdot CH_3OH$, whose methanol of crystallization is lost in storage. It exhibits λ_{max}^{EtOH} 388 mμ (ε 48,000), 294 (27,100), 286_{sh} (24,600), 234 (10,200), 204 (17,000); ν_{max}^{KBr} 3500, 1702, 1689, 1654, 1626, 1562, 1531, 1452, 1405, 1249, 1094, 1069, 999, 821, 811 cm^{-1}; NMR (Fig 1); and contains one methoxyl group (Zeisel) and about 6 double bonds (catalytic hydrogenation). Acetylation of the compound afforded a monoacetate (II), m.p. 99-101°, whereas p-nitrobenzoylation gave a mono- (III), m.p. 178-178.5°, and a di-p-nitrobenzoate (IV), m.p. 269-271°.

Permanganate oxidation of citreoviridin (I) in pyridine afforded a carboxylic acid (V), m.p. 216°. The acid, $C_8H_8O_5$, exhibits λ_{max}^{EtOH} 293 mμ (ε 6,400) and pKa' 2.8. The NMR spectrum of its methyl ester, m.p. 131-132°, shows the presence of one methyl (2.25 p.p.m., s)*, two

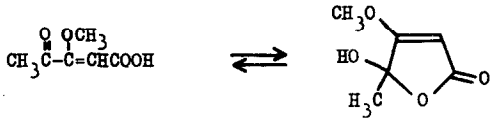
* 60Mc; solvent $CDCl_3$; p.p.m. from internal tetramethylsilane; s = singlet, d = doublet, q = quartet, br = broad.

methoxyl groups (3.92, s, 6H), one of which is attributable to a methoxycarbonyl group, and one vinyl proton (5.78, s). The infrared (1720, 1630, 1558 cm^{-1}) and the ultraviolet spectra (λ_{max}^{EtOH} 294 mμ, ε 6,030) of the methyl ester suggest that it is an α-pyrone, and not a γ-pyrone, derivative (6). Thus, the acid (V) can be represented by a partial formula (Va). That the 6-position in (Va) is substituted is evident from the signal of vinyl proton (5.78), since signals of C_6-proton in α-pyrone derivatives are usually around 7.5 p.p.m. (7). When a limited amount of permanganate was used, the corresponding aldehyde, m.p. 140-141°, was obtained.



(Va)

Ozonolysis of citreoviridin (I) gave glyoxal and diacetyl, both of which were identified as their 2,4-dinitrophenylhydrazone. The p-nitrobenzoate (III), on ozonization in dichloromethane at -70° followed by chromatography on silica-gel, gave an oily substance (VI), which exhibits λ_{max}^{EtOH} 220 mμ; ν_{max} 3340, 1746, 1643 cm^{-1}; NMR: 1.65 (s, 3H), 3.98 (s, 3H), 5.10 (s, 1H), 5.65 (br, 1H); and on treatment with a solution of 2,4-dinitrophenylhydrazine in hydrochloric acid gave diacetyl 2,4-dinitrophenylhydrazone. Thus, (VI) can be condidered as a precursor of diacetyl and the physical constants suggest its structure as (VIb), which is in equilibrium with the open-chain acid (VIa).



(VIa) (VIb)

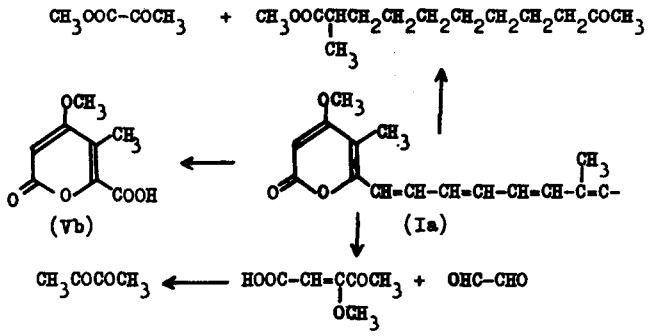
Partial catalytic hydrogenation, followed by ozonization, of citreoviridin (I) afforded methyl pyruvate (characterized as its 2,4-dinitrophenylhydrazone) and an oily methyl ester (VII). The latter (VII) has a molecular weight 228 (mass spectrometry), showed a positive iodoform test, and gave a 2,4-dinitrophenylhydrazone, $C_{19}H_{28}O_6N_4$, m.p. 82-82.5°. The NMR spectrum of (VII) shows the following signals:

0.96 p.p.m. (d, 3H; secondary methyl), 1.38 (br, 12H; saturated C-H), 2.30 (s, 3H; CH_3CO-), 2.50 (br, 3H; -COCH-) and 4.50 (s, 3H; CH_3O-). The structure of the methyl ester (VII) must be represented by (VIIa), in which the methoxycarbonyl and the methyl groups are vicinal to each other since they must have been present in the pyrone moiety.

$$CH_3OOCCH(CH_3)CH_2CH_2CH_2CH_2CH_2CH_2CH_2COCH_3$$

(VIIa)

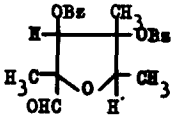
The above results indicate the partial formula (Ia) for citreoviridin. The ultraviolet spectrum of citreoviridin (I)(388 mμ, ε 48,000) suggests that the pyrone chromophore is in conjugation with a polyene moiety, and the NMR spectrum (Fig. 1) also supports the partial formula (Ia).

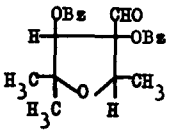


Citreoviridin di-p-nitrobenzoate (IV), when being ozonized in dichloromethane, gave an saturated aldehyde (VIII), $C_8H_{12}O_4(COC_6H_4NO_2)_2$, m.p. 205-208°. The NMR spectrum of the aldehyde suggests the presence of the following groups: (a) CH_3-$\overset{|}{C}$-O- (1.29 p.p.m., s, 3H); (b) CH_3-$\overset{|}{C}$-O- (1.70, s, 3H); (c) CH_3-CH-O-C* (1.53, d, J=6.5 cps, 3H; 4.46, q, J=6.5 cps, 1H); (d) -HC-OBz (6.46, s, 1H; 8.36, s, 4H, Bz = $-C_6H_4NO_2$-p); (e) -$\overset{|}{C}$-OBz (8.06, 8.21, 8.29, 8.44, AB type quartet, 4H); (f) -$\overset{|}{C}$-CHO (9.60, s, 1H). The carbon atoms bearing the same mark, (*) or (°), may be the same carbon atom. All of the corresponding signals, except that of the aldehydic proton, is present in the NMR spectrum of citreoviridin di-p-nitrobenzoate (IV). Since on p-nitro-

benzoylation of citreoviridin (I) the corresponding quartet to that in group (c) is not much shifted as expected from the transformation of CH_3CH-OH to CH_3CH-OBz, the oxygen atom in (c) must link with two carbon atoms. To accommodate the above groups into the molecular formula $C_8H_{12}O_4(COC_6H_4NO_2)_2$, only the following four formulas, VIIIa ~VIIId, are possible.

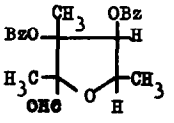
(VIIIa)

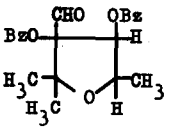




(VIIIb)

(VIIIc)

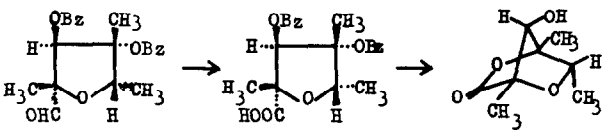




(VIIId)

Since the quartet in (c) and singlet in (d) show no indication to couple mutually (also in the NMR of the lactone (X); see below), the formulas VIIIc and VIIId can be excluded.

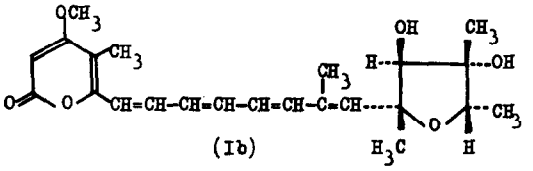
The aldehyde (VIII) was oxidized with permanganate in pyridine to the corresponding acid (IX), $C_8H_{12}O_5(COC_6H_4NO_2)_2$, m.p. 230-233°. Hydrolysis of the acid (IX) with base, followed by acidification, afforded an oily five-membered lactone (X)(IR: 1798 cm^{-1}), the NMR of which indicates no skeletal rearrangement or dehydration: 1.21 p.p.m. (d, J=6 cps, 3H), 1.38 (s, 3H), 1.48 (s, 3H), 2.80 (br, 1H; hydroxyl), 3.72 (s, 1H), 4.33 (q, J=6 cps, 1H). From this result, the aldehyde (VIII) must have the structure (VIIIa), whose configuration is deduced as (VIIIe) from the following evidence: (a) the 4-hydroxyl group is cis to the 2-aldehyde group since the corresponding acid forms a lactone (X); (b) two vicinal hydroxyl groups are trans to each other since citreoviridin (I) consumes periodate only very slowly (in acidic aqueous methanol, 0.0 mole after 11 hrs., 0.8 mole after 100 hrs.); (c) that the C_5-proton is trans to the C_3-proton is deduced from the fact that the p-nitrobenzoyl group at 4-



(VIIIe) (IX) (X)

position affects the NMR signal of the C_3-proton very strongly (1.31 p.p.m.), whereas only small shift (0.26 p.p.m.) is observed for the C_5-proton (III → IV).

The structure of citreoviridin is now represented by the formula (Ib), which is reasonable from the biogenetic point of view.



(Ib)

It may be worth to note that some of the NMR signals of citreoviridin (I) are strongly shifted by change of the solvent from deuterochloroform to benzene as shown in figure 1. The signals, which move to higher field by the solvent change, belong to the pyrone moiety, whereas slight down-field shifts were observed on the signals belonged to the tetrahydrofuran moiety. The pyrone nucleus is planar and absorbs benzene molecules, anisotropy of which may shift the signals of the pyrone group to higher field.

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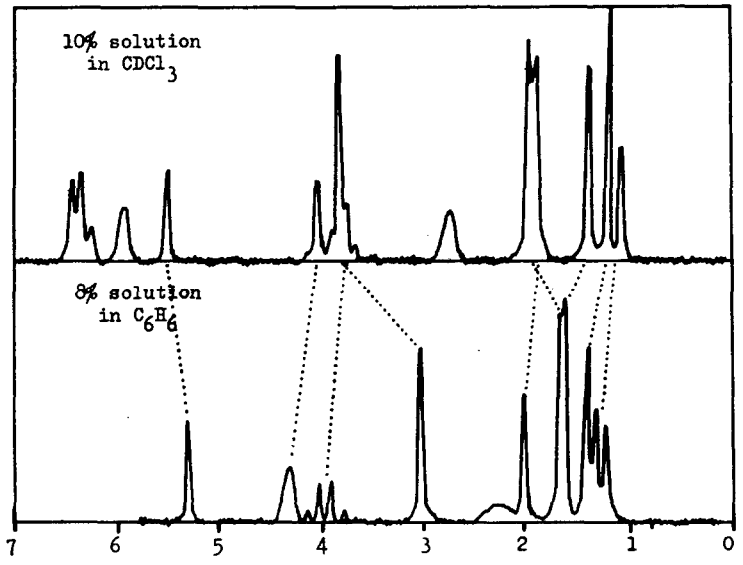

Fig 1. NMR spectra of citreoviridin (I)
at 60 Mc., p.p.m. from internal TMS

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