Tetrahedron Letters No. 36, pp 3789 - 3792, 1972. Pergamon Press. Printed in Great Britain.

Structures of Viridominic Acids A and B, New Chlorosis-inducing Metabolites of a Fungus

H. Kaise and K. Munakata

Department of Agricultural Chemistry, Nagoya University, Nagoya, Japan.

T. Sassa

Department of Agricultural Chemistry, Yamagata University, Tsuruoka, Yamagata, Japan.

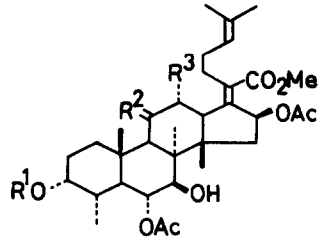
(Received in Japan 17 July 1972; received in UK for publication 1 August 1972)

Viridominic acids A, B and C (VA-A, -B and -C) are chlorosis-inducing substances, isolated from the culture filtrate of a fungus No. 501-7Y[1] (identified as Cladosporium sp.). In addition, cephalosporin P_1 ($C\text{-}P_1$)[2] (1) was isolated as a minor product from the culture filtrate[3]. The structure of VA-C was assigned previously as (11)[4]. It was very interest to resolve the structures of VA-A and -B, because these compounds have more stronger chlorosis-inducing activities than VA-A and $C\text{-}P_1$. The activities of VA-A and -B are more ten-fold higher than $C\text{-}P_1$ and near one hundred-fold higer than VA-C[3]. We now wish to report the structures of VA-A and -B as (III) and (IV), respectively.

Viridominic acid A (III), $C_{33}H_{48}O_9$, mp 151-155°C, $[\alpha]_D^{22}$ +49°(c, 0.1, MeOH), is a carboxylic acid and its IR and NMR spectral patterns were closely resemble to those of $C\text{-}P_1$ and VA-C (Table I). VA-A contained isopentenyl [δ^{CDCl_3} 1.61, 1.69 each 3H, s; 5.09, 1H, br.t; methly ester (V), m/e 533 (M^+-69)], α,β-unsaturated carboxylic acid [λ_{max}^{EtOH} 220(sh) nm, (ε 6,500); (V), δ^{CDCl_3} 3.64, 3H, s], hydroxyl (ν_{max}^{KBr} 3450 cm^{-1}) and two acetoxyl (δ^{CDCl_3} 1.95, 2.03 each 3H, s) groups.

The UV spectrum of (III) showed a shoulder at 290 nm (ε 103), which suggested that VA-A has a keto-group. The existence of a ketone in (III) was confirmed by sodium borohydride reduction of methyl ester (V, CD curve: positive Cotton effect, $[\theta]_{299}$ +5,770) which afforded a dihydro-ol [VI, mp 119-121°C, m/e 544 (M^+-60), δ^{CDCl_3} 4.48, br.s, overlapped with H-6].

Oxidation of (V) with Jones reagent afforded diketoVA-A [VII, mp 124-127.5°C, m/e 544 (M^+-60), λ_{max}^{EtOH} 287(sh) nm (ε 500)]. The IR, NMR, UV and mass spectra of (VII) agreed with those of the triketone obtained from VA-C by oxidation with the same reagent. Thus the structure of VA-A was determined as (III), since the presence of C-3 and -7 hydroxyl groups were indicated



(I) $R^1=R^3=R^4=H$, $R^2=H_2$

(II) $R^1=R^3=R^4=H$, $R^2=\beta$-OH,H

(III) $R^1=R^3=R^4=H$, $R^2=O$

(IV) $R^1=R^4=H$, $R^2=O$, $R^3=OH$

(V) $R^1=R^3=H$, $R^2=O$, $R^4=Me$

(VI) $R^1=R^3=H$, $R^2=\alpha$-OH,H, $R^4=Me$

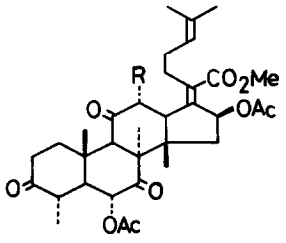
(VIII) $R^1=R^3=H$, $R^2=H_2$, $R^4=Me$

(IX) $R^1=H$, $R^2=O$, $R^3=OH$, $R^4=Me$

(XII) $R^1=H$, $R^2=\alpha$-OH,H, $R^3=OH$, $R^4=Me$

(XIII) $R^1=H$, $R^2=H,\alpha$-O(Me)(Me), $R^4=Me$, $R^3=$ O(Me)(Me)

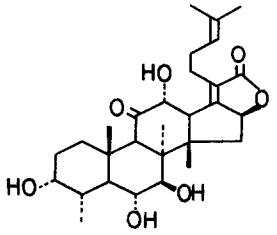
(XIV) $R^1=Ac$, $R^2=\alpha$-OH,H, $R^3=OAc$, $R^4=Me$



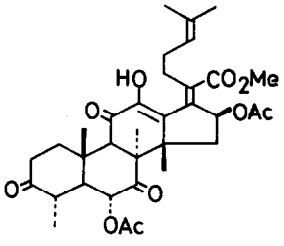
(VII) R=H

(X) R=OH

(XI) R=OAc



(XVI)



(XV)

by the comparison of the NMR spectrum of (V) with those of methyl esters of (I) and (II) (Table I).

Viridominic acid B (IV), $C_{33}H_{48}O_{10}$, amorphous, $[\alpha]_D^{17}$ +64° (c,0.37, MeOH), also contains α,β-unsaturated carboxylic acid [λ_{max}^{EtOH} 220(sh) nm (ε 7,300); methyl ester (IX), δ^{CDCl_3} 3.64, 3H, s], isopentenyl [δ^{CDCl_3} 1.58, 1.65 each 3H, s, 5.14, br.t; (IX), m/e 594 (M^+-69)], hydroxyl (ν_{max}^{KBr} 3450 cm^{-1}) and two acetoxyl (δ^{CDCl_3} 1.98, 2.03 each 3H, s) groups. The NMR and IR spectra of (IV) were closely similar to those of (I), (II) and (III), and it suggested that (IV) has the same carbon skeleton and functional groups to another three metabolites of this fungus. Oxidation of (IX) with chromium trioxide-pridine then acetylation afforded a diketo-acetate [XI, mp 236-240°C, m/e 596 (M^+-60), λ_{max}^{EtOH} 290(sh) nm (ε 260), δ^{CDCl_3} 1.97, 2.16 and 2.21 each 3H, s] suggesting that VA-B has another hydroxyl group than C-3 and -7 ones.

The UV spectrum of VA-B [IV, 290(sh) nm (ε 151)], and a dihydro-ol[XII, m/e 588 (M^+-32), 560 (M^+-60)] formation by sodium borohydride reduction showed the presence of a keto-group in

Table I. NMR Signals of Methyl Esters of Viridominic Acids A, B and C (VA-A, -B and -C) and Cephalosporin P_1 ($C\text{-}P_1$) in $CDCl_3$

	VA-A	VA-B	VA-C*	$C\text{-}P_1$
H- 3	3.70, br.s	3.73, br.s	3.49, br.s	3.69, br.s
H- 6	4.48, d (11)	4.45, d (10)	4.62, d (12)	4.53, d (11)
H- 7	3.50, s	3.50, s	3.26, s	3.48, s
H- 9	3.28, s	3.39, s		
H-11			3.70, m	
H-12		4.28, d (11)		
H-16	5.84, d (8)	5.73, d (8)	5.68, d (8.5)	5.78, d (8.5)
H-24	5.05, br.t (8)	5.12, br.t (7.5)	5.11, br.t (7)	5.07, br.t (7)
sec. Me	0.92, d (7)	0.92, d (6.5)	0.77, d (6.5)	0.89, d (7)
tert. Me	1.03	0.90	0.99	1.03
	1.28	1.32	1.07	1.16
	1.47	1.56	1.16	1.16
vinyl Me	1.60	1.58	1.57	1.58
	1.67	1.65	1.64	1.66

* in D_6-DMSO

(IV). The presence of the keto- and hydroxy-groups at vicinal carbon atoms was suggested by formation of an acetonide (XIII, δ^{CDCl_3} 1.26, 1.27 each s) from (XII). The NMDR spectrum of the diacetate (XIV) obtained from (XII) confirmed the arrangement of hydroxyl (produced by sodium borohydride reduction from the keto-group) and acetoxyl groups at vicinal carbon atoms.

The location of α-hydroxy ketone group at C-11,12 was confirmed by Jones oxidation of (IX), which afforded a mixture (ca. 1:1) of the diketone (X) and an enol-diketone (XV) [positive to ferric chloride test; m/e 554 (M^+-60), 552 (M^+-60); λ_{max}^{EtOH} 330 nm (calcd. value according to Fieser's rule[5]: 333 nm); δ^{CDCl_3} 6.78, s, disappeared by addition of D_2O]. These two compounds could not be separated by chromatographic techniques.

The presence of C-16 acetoxyl and C-21 carboxylic acid, and the cis relationship of C-16 and -21 were confirmed by yhe formation of a lactone [XVI, mp 242-244°C, λ_{max}^{EtOH} 217 nm (ε 12,000), ν_{max}^{KBr} 1745 cm^{-1}, $\delta^{CDCl_3+D_6\text{-DMSO}}$ 5.01, d.d] from VA-B by alkali-hydrolysis. That the keto-group of VA-B is located at C-11 was suggested by comparison of the NMR spectrum of (IX) with those of $C\text{-}P_1$ methyl ester (VIII) and VA-A methyl ester (V) and solvent-shifts of methyl groups of (IX) in the NMR spectrum ($CDCl_3$ and C_6D_6). As compared with the NMR spectrum of (VIII), one of the tert. methyl group of (IX) shifted to upfield (0.13 ppm) and another two downfield (0.18 and

0.41 ppm). These shifts of methyl groups of (V) were observed in the NMR spectrum. The NMR spectrum of VA-B dihydro-ol (XII) showed tert. methyl groups at δ 1.18, 1.28 and 1.28. These chemical shifts were almost similar to those of VA-A dihydro-ol (VI, δ 1.16, 1.26 and 1.30). One of Δ value[6] (δ^{CDCl_3}-$\delta^{C_6D_6}$) of the tert. methyl groups of (IX) showed negative (-0.03) and this evidence, combined with above NMR data, suggested that VA-B has C-11 keto-group, because if VA-B has C-12 keto-group, all of Δ values of tert. methyl groups should show positive. The mass spectrum of diketoVA-B methyl ester (X) showed relatively intense peaks at m/e 243, 229, 223, 212 and 123. It is considered most reasonable that these ions are derived from McLafferty rearrangement ions by α-cleavage of C-11 keto-group[4]. The CD curve of (IX, positive Cotton effect $[\theta]_{298}$ +6,670) was almost superimposable with that of (V). These data supported that VA-B has C-11 keto-group.

The chemical shifts and splitting patterns of H-3, -6, -7 and -16 in the NMR spectrum of (IV) and (XVI) indicated that C-3, -6, -7 and -16 substituents are the same configurations as other metabolites (I), (II) and (III). The coupling constant (11 Hz) of H-12 showed the equatorial orientation of C-12 hydroxyl group. Thus we assigned the structure of VA-B as (IV).

Acknowledgements: The authers are indebted to Professor J. Tanaka of fakulty of science of this university for measurement of CD spectrum, and Dr. K. Tsubaki of Takeda Chemical Industries, Ltd. for identification of the fungus. We are also grateful to Dr. S. Marumo of this laboratory for his useful advice.

References

1) H. Kaise, Y. Ogawa, T. Sassa and K. Munakata, Agr. Biol. Chem., 34, 1760 (1970).

2) T. G. Halsall, Sir E. R. H. Jones, G. Lowe and E. E. Newall, Chem. Comm.,685 (1966). P. Oxley, ibid., 729 (1966), T. S. Chou and J. Eisenbrau, Tetrahedron Letters, 409 (1967).

3) H. Kaise, Y. Ogawa, T. Sassa and K. Munakata, Agr. Biol. Chem., 36, 120 (1972).

4) H. Kaise, K. Munakata and T. Sassa, Tetrahedron Letters, 199 (1972).

5) A. L. Scott, "Introduction of the Ultraviolet Spectra of Natural Products.", Pargamon Press, New York, 1964, p. 55.

6) D. H. Williams and N. S. Bacca, Tetrahedron, 21, 2021 (1965).