Tetrahedron Letters No. 3, pp 199 - 202, 1972. Pergamon Press. Printed in Great Britain.

STRUCTURE OF VIRIDOMINIC ACID C, A NEW STEROIDAL METABOLITE OF A FUNGUS HAVING CHLOROSIS-INDUCING ACTIVITY

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 6 December 1971; received in UK for publication 14 December 1971)

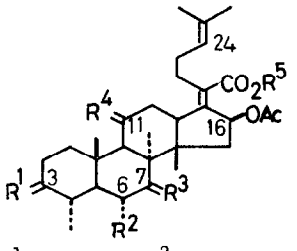
We previously reported the isolation and biological activities of viridominic acid A and C[1], having chlorosis-inducing activity against higher plants, from the culture filtrate of the fungus No. 501-7Y (genus and species unidentified). In addition, cephalosporin P_1[2] was isolated from the culture filtrate as a minor product, which also induced chlorosis of higher plants. It is known as antibiotic possessing protostane skeleton[3]. In this paper we wish to report the chemical structure of viridominic acid C (VA-C) (I), a new steroidal metabolite of fungus in structurally relating to cephalosporin P_1 (II).

VA-C (I), $C_{33}H_{50}O_9$, mp 168-171°C, $[\alpha]_D^{25}$ +38° (c, 0.1, MeOH), ν_{max}^{KBr} 3450, 1715(br), 1265 and 915 cm^{-1}, exhibits quite similar NMR (Table 1) and IR spectral patterns to those of cephalosporin P_1 (II). VA-C (I) showed UV absorption maximum at 220 nm (ε 8,100) and was methylated with ethereal diazomethane to give a monomethyl ester (III), mp 186.5-187.5°C, $\delta^{D}6^{-DMSO}$ 3.56, 3H, s. VA-C was treated with 1N aq. NaOH at room temperature to give a deacetyl derivative (IV). mp 196-198°C, $\delta^{D}6^{-DMSO}$ 1.88, 3H, s; 5.65, 1H, d, J.8Hz, H-16; 3.26, 1H. d, J,13Hz, H-6. Further treatment of (IV) with 1N aq. NaOH afforded a lactone (V), $C_{29}H_{44}O_6$, mp 248-250°C, λ_{max}^{EtOH} 222 nm (ε 14,100), ν_{max}^{KBr} 1740 cm^{-1}, $\delta^{D}6^{-DMSO}$ ca. 5.1, 2H, H-16, 24. Above data indicated that (V) no longer contained an acetoxyl group, and the lactone was α,β-unsaturated γ-lactone. Thus (I) has a partial structure $-\overset{COOH}{\underset{|}{C}}=C-\overset{OAc}{\underset{|}{C}}-$.

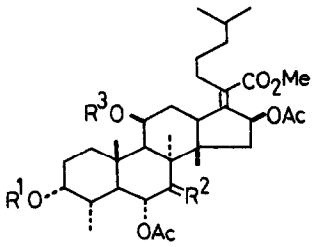
The NMR spectrum (Table 1) of (I) displayed the presence of isopropylidene group and one vinylic proton (δ 5.05, H-24). Catalytic hydrogenation of (III) over 10% Pd/C in EtOH afforded a dihydro ester (VI), mp 180.5-182.5°C, m/e 606 (M^+), λ_{max}^{EtOH} 220 nm (ε 8,100). In the NMR spectrum of (VI), signal of isopropyl group ($\delta^{D}6^{-DMSO}$ 0.84, 6H, d, J.7Hz) was newly appeared and that of vinylic proton was disappeared. These data suggested the existence of $-CH_2-CH=C\begin{matrix}CH_3\\CH_3\end{matrix}$ in (I)

Table 1. The NMR Spectra of Viridominic Acid C and Cephalosporin P_1 in $CDCl_3$+D_6-DMSO (5:1) (ppm from TMS)

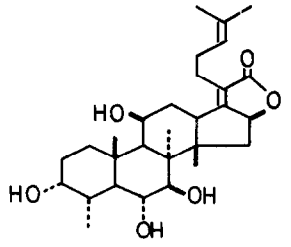
	sec. Me	tert. Me	vinyl Me	H-3	H-6	H-7	H-16	H-24
VA-C (I)	0.82 d,(7)	0.98, 1.15, 1.19	1.55, 1.61	3.51 br,s	4.51 d,(10)	3.30 s	5.74 d,(7.5)	5.05 br,t(7.5)
C-P_1 (II)	0.80 d,(6.5)	0.99, 1.09, 1.09	1.52, 1.60	3.52 br,s	4.50 d,(10)	3.38 s	5.67 d,(8)	5.00 br,t(7.5)



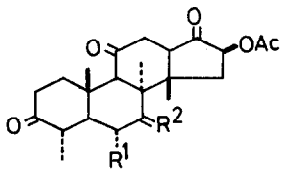
(I) R^1=α-OH,β-H, R^2=OAc,
R^3=R^4=β-OH,α-H, R^5=H
(II) R^1=α-OH,β-H, R^2=OAc, R^3=β-OH,α-H,
R^4=H_2, R^5=H
(III) R^1=α-OH,β-H, R^2=OAc,
R^3=R^4=β-OH,α-H, R^5=Me
(IV) R^1=R^2=α-OH,β-H,
R^3=R^4=β-OH,α-H, R^5=H
(VII) R^1=α-OH,β-H, R^2=OAc, R^3=β-OH,α-H,
R^4=β-OH,α-H, R^5=Me
(X) R^1=α-OH,β-H, R^2,R^3=$>C<^{CH_3}_{CH_3}$,
R^4=β-OH,α-H, R^5=H
(XI) R^1=R^3=R^4=O, R^2=OAc, R^5=Me
(XV) R^1=R^4=α-OH,β-H, R^2=R^5=H, R^3=H_2



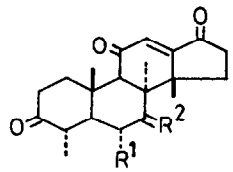
(VI) R^1=R^3=H, R^2=β-OH,α-H
(VIII) R^1=R^3=Ac, R^2=β-OH,α-H
(IX) R^1=R^3=Ac, R^2=O



(V)



(XII) R^1=OAc, R^2=O
(XIV) R^1=H, R^2=H_2



(XIII) R^1=OAc, R^2=O
(XVI) R^1=H, R^2=H_2

The peak of m/e 535 (M^+-69) in the mass spectrum of (III) supported the above partial structure.

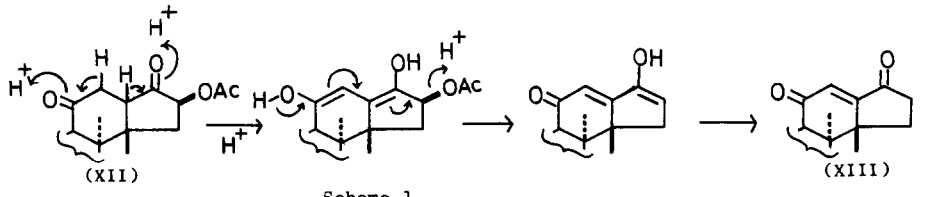
The NMR spectrum of (I) (Table 1) displayed the presence of three tertiary methyl, one secondary methyl and two secondary acetoxyl groups. The NMR spectrum of methyl ester (III) in D_6-DMSO showed three doublets (δ 4.83, J,5Hz; 4.34, J,7Hz; 3.14, J,3.5Hz) and these signals were disappeared by addition of D_2O. This evidence pointed to the presence of three secondary hydroxyl groups in (I). Acetylation of (III) with acetic anhydride and pyridine afforded a diacetate (VII), δ^{CDCl_3} 2.09, 9H; 1.96, 3H; 4.87, 1H, br,s, H-3; 5.08, 2H, m, H-11, 24. The NMR spectrum of dihydro diacetate (VIII), m/e 630 (M^+-60), δ^{CDCl_3} 0.86, 6H, d, J,5.5Hz, $\nu_{max}^{CCl_4}$ 3500 cm^{-1},

obtained by catalytic hydrogenation of (VII) on 10% Pd/C showed a isolated signal of H-11 at δ 5.06 (m, $W_{1/2}$ 23 Hz), which was overlapped with vinylic proton (H-24) in that of (VII). Oxidation of (VIII) with Jones' reagent at 0°C afforded monoketone (IX), m/e 688 (M^+). The IR spectrum of (IX) had no absorption near 3500 cm^{-1}.

Deacetyl edrivertive (IV) was treated with acetone and catalitic amount of p-TsOH and 2,2-dimethoxyacetone to afford an acetonide (X), δ^{CDCl_3} 1.32, 1.36 each 3H, s; 3.34, 1H, d, J,9.5Hz, H-7; 3.53, 1H, t, J,9.5Hz, H-6, which indicated that the hydroxyl and acetoxyl groups in (I) were located at vicinal carbon. Secondary methyl group in deacetyl derivative (IV) showed the large downfield shift (0.24 ppm) in the NMR spectrum, compared with that of (I). Thus the secondary methyl and acetoxyl group in (I) were located at 1,3 position, and probably C-OAc bond is nearly parallel to C-Me bond[4].

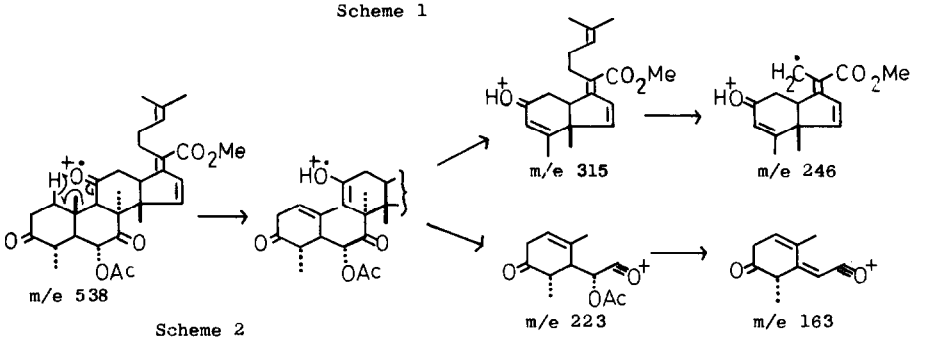
These physical and chemical data suggested that VA-C (I) has the same carbon-skeleton and functional groups at the same positions of $C\text{-}P_1$ (II), and (I) has more additional secondary hydroxyl group. The position of this hydroxyl group was determined as followed. Methyl ester (III) was oxidized with Jones' reagent to afford a triketone (XI) mp 125-128°C, m/e 538 (M^+-60), δ^{CDCl_3} 1.29, 3H, d, J,6.5Hz, C_4-Me. Ozonolysis of (XI) in CH_2Cl_2 at dry ice temperature followed by decomposition of ozonide with zinc dust and acetic acid afforded acetone (its 2,4-DNP, mp 129°C) and an ene-dione (XIII), pale yellow crystal, mp 230-233°C, $C_{23}H_{28}O_6$. The UV spectrum of (XIII) showed absorption maximum at 256 nm (ε 9,300), which was characteristic to ene-dione chromophore[5]. This was supported from the carbonyl absorptions in the IR spectrum ($\nu_{max}^{CHCl_3}$ 1720, 1681 cm^{-1}). The NMR spectrum showed that (XIII) had only one acetoxyl group (δ^{CDCl_3} 2.23, 3H, s; 5.37, 1H, d, J,12.5Hz) and one vinylic proton (δ^{CDCl_3} 6.23, 1H, s, H-12). Arigoni et al. reported that the ozonolysis product (IV) of fusidic acid (XV) was converted to the ene-dione (XVI) by treatment with base[6]. The formation of (XIII) was interpreted by the anologous reaction scheme, namely the ozonolysis product (XII) was transformed into the ene-dione by acetic acid with elimination of α-acetoxyl group as shown in scheme 1.

Thus the another hydroxyl group mrst be located at C-11. This was supported by the mass spectrum of triketone (XI). It showed relatively intense peaks at m/e 315, 246, 223 and 163. It is considered most reasonable that these ions are derived frcm McLafferty rearrangement ion by α-cleavage of C-11 ketone (scheme 2). The large half-band width of H-11 (23 Hz) in the NMR spectrum of (VIII) and the ease of acetylation of C-11 hydroxyl group indicated that the configuration of this group is equatorial. It is known that the C-11 equatorial hydroxyl group is acety-



(XII) (XIII)

Scheme 1



m/e 538 m/e 315 m/e 246 m/e 223 m/e 163

Scheme 2

lated easily[7] but axial one resists to acetylation[5].

As shown in Table 1, the chemical shifts and splitting patterns of H-3, -6, -7 and -16 in the NMR spectrum of (I) were almost superimporsable to those of C-P_1 (II). This suggested that the hydroxyl and acetoxyl groups in (I) gave the same configurations to (II). The large down-field shift (0.47 ppm) of the signal of C-4 methyl group in the NMR spectrum of triketone (XI) compared with that of (I) demonstrated that the secondary methyl group in (I) is the equatorial configuration. Thus we concluded that viridominic acid C is 3α,7β,11β-trihydroxy-6α,16β-diacetoxyfusida-17(20)[16,21-cis],24-dien-21-oic acid (I).

Acknowledgements: The authers are indebted to Dr. T.G. Halsall of the Dyson Perrins Laboratories of Oxford University, Dr. T.S. Chou of the Lilly Research Laboratories of Elly Lilly and Company and Dr. P. Oxley of Boots Pure Company Ltd. for their generous supply of authentic sample of cephalosporin P_1. We are also grateful to the members of Fujisawa Pharmaceutical company Ltd. and Kaken Kagaku Company Ltd. for their preparing of crude extracts of the fungal broth.

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) T. Hattori, H. Igarashi, S. Iwasaki and S. Okuda, Tetrahedron Letters, 1023 (1969).

(4) T. Okamoto and Y. Kawazoe, Chem. Phar. Bull. (Tokyo), 11, 328, 643 (1963).

(5) W.O. Godtfredsen and S. Vangedal, Tetrahedron, 18, 1029 (1962).

(6) D. Arigoni, W. von Daehne, W.O. Godtfredsen, A. Marquet and A. Malera, Experientia, 19, 521 (1963)

(7) T. Murata, M. Sinohara, T. Hirata and M. Miyamoto, Tetrahedron Letters, 849 (1968).