Pergamon

Tetrahedron Letters, Vol. 37, No. 51, pp. 9219-9220, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)02187-9

Trichothecinols A, B and C, Potent Anti-Tumor Promoting Sesquiterpenoids from the Fungus *Trichothecium roseum*

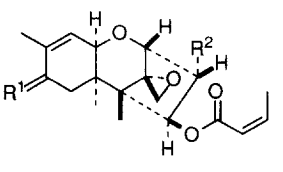
Akira Iida,[a]* Kazuhide Konishi,[a] Hiroki Kubo,[a] Kiyoshi Tomioka,[a] Harukuni Tokuda[b] and Hoyoku Nishino[b]

[a] Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan

[b]Department of Biochemistry, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602, Japan

Abstract: Three new trichothecenes, trichothecinols A (**1**), B (**2**) and C (**3**) were isolated from the fungus *Trichothecium roseum* and unambiguiously characterized on the basis of spectroscopic and chemical evidence. These exhibited potent inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) activation induced by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA).

In our continuing studies on biologically active fungal metabolites, three new sesquiterpenoids, trichothecinols A (**1**), B (**2**) and C (**3**), were isolated from *Trichothecium roseum* (TMI-32358), supplied from The Tottori Mycological Institute (Tottori, Japan), together with an antifungal antibiotic, trichothecin[1] (**4**, 12,13-epoxy-4β-hydroxytrichothec-9-en-8-one 4-isocrotonate). In addition, we found that they exhibit potent anti-tumor promoting activitiy, which is evaluated through their inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) activation induced by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells.[2] We describe the structure determination and anti-tumor promoting activity of **1-4**.



Trichothecinol A (**1**: R^1 = O, R^2 = OH)
B (**2**: R^1 = α-OH, β-H, R^2 = H)
C (**3**: R^1 = α-OH, β-H, R^2 = OH
Trichothecin (**4**: R^1 = O, R^2 = H)

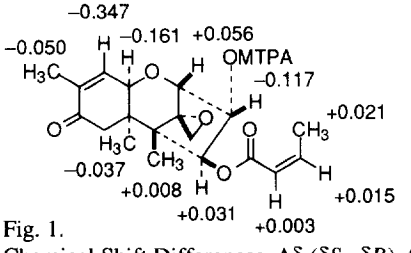


Fig. 1. Chemical Shift Differences, Δδ (δ*S* - δ*R*), for the (*S*)- and (*R*)-MTPA Esters Derived from **1**.

The IR data and molecular weight of **1** ($C_{19}H_{24}O_6$)[3] suggested that **1** is a hydroxy form of **4** ($C_{19}H_{24}O_5$).[1,4] Detailed analysis of the NOE data of **1** and the modified Mosher's method[5] allowed us to assign the absolute stereostructure of **1** as 12,13-epoxy-3α,4β-dihydroxytrichothec-9-en-8-one 4-isocrotonate (Fig. 1). The NOE data and molecular weights of **2** ($C_{19}H_{26}O_5$) and **3** ($C_{19}H_{26}O_6$) revealed the relative structures of **2** and **3** as shown, which are the 8-hydroxy forms[6, 7] of **4** and **1**, respectively. Reduction of the C-8 ketone of **4** by $NaBH_4$ afforded a mixture of two separable epimeric alcohols (5:2). The NMR data of the major product were identical with those of **2**. In addition, its specific rotation ($[\alpha]_D^{18}$ −14.1 (c 0.3, MeOH)) and retention time on HPLC were in good agreement with those of **2**.[6] Therefore, the absolute structure of **2** was established as shown. Similarly, the absolute structure of **3** was determined as shown by reducing **1** to the corresponding diols, of which major product ($[\alpha]_D^{18}$ +28.6 (c 0.2, MeOH)) was identical with **3**.[7]

The inhibitory effects of **1-4** and β-carotene on EBV-EA activation induced by TPA are presented in Table 1. The trichothecenes **1-4** were quite potent anti-tumor promoters[8] in comparison with β-carotene, a vitamin A precursor that has been most intensively studied in cancer prevention[9,10] using animal models. At the

same time, their cytotoxicity against Raji cells was very low. A study on the structure-activity relationship is now in progress using analogues derived from the natural trichothecenes.[11]

Table 1. Inhibitory Effects of Trichothecenes on TPA-induced EBV-EA Activation.

	% to control (% viability) at concentration (mol ratio/TPA)					
	1000	500	100	10	1	0.1
1	0 (70)	0	0	0	32	80
2	0 (70)	0	0	63	82	100
3	0 (70)	0	0	0	33	81
4	0 (70)	0	0	23	42	87
β-carotene	9 (70)	34	82	100	100	-

Values represent relative percentages to the positive control value. TPA (32 pmol, 20 ng)=100%. Values in parentheses are viability percentages of Raji cells.

REFERENCES and NOTES

1. Freeman, G. G.; Gill, J. J.; Waring, W. S. *J. Chem. Soc.* **1959**, 1105-1132; Godteredsen, W. O.; Vangedal, S. *Acta. Chem. Scand.* **1965**, *19*, 1088-1102.
2. Ito, Y.; Yanase, S.; Fujita, J.; Harayama, T.; Takashima, M.; Imanaka, H. *Cancer Lett.* **1981**, *13*, 29-37; Tokuda, H.; Ohigashi, H.; Koshimizu, K.; Ito, Y. *ibid*, **1986**, *33*, 279-286.
3. Data for **1**: HR-CIMS *m/z* 349.1656 (MH^+, calcd mass=349.1651); $[\alpha]_D^{18}$ +81.5 (c 0.7, MeOH); IR ($CHCl_3$) ν_{max} 3450, 1720, 1680 cm^{-1}; UV λ_{max}^{MeOH} 218 nm (ε=1.4 x 10^4); ^{1}H-NMR (300 MHz, $CDCl_3$), δ 3.78 (d, *J* = 5.0 Hz, H-2), 4.29 (ddd, *J* = 2.6, 3.0, 5.0 Hz, H-3β), 4.99 (d, *J* = 3.0 Hz, H-4), 2.31 (dd, *J* = 1.6, 15.2 Hz, H-7α), 2.95 (dd, *J* = 1.2, 15.2 Hz, H-7β), 6.595 (dq, *J* = 1.4, 5.8 Hz, H-10), 4.41 (dd, *J* = 0.8, 5.8 Hz, H-11), 2.81 (d, *J* = 3.9 Hz, H-13 *pro-R*), 3.08 (d, *J* = 3.9 Hz, H-13 *pro-S*), 0.77 (s, 5-Me), 1.05 (d, *J* = 1.2 Hz, 6-Me), 1.84 (dd, *J* = 0.8, 1.4 Hz, 9-Me), 3.50 (d, *J* = 2.6 Hz, 3-OH), 5.88 (dq, *J* = 1.8, 11.5 Hz, H-2'), 6.45 (dq, *J* = 7.3, 11.5 Hz, H-3'), 2.17 (dd, *J* = 1.8, 7.3 Hz, 3' Me); ^{13}C-NMR (75 MHz, $CDCl_3$), δ 79.30 (d, C-2), 78.77 (d, C-3), 83.17 (d, C-4), 48.87 (s, C-5), 44.39 (s, C-6), 42.03 (t, C-7), 198.46 (s, C-8), 137.77 (s, C-9), 137.24 (d, C-10), 70.97 (d, C-11), 64.50 (s, C-12), 46.63 (t, C-13), 5.90 (q, C-14), 18.40 (q, C-15), 15.33 (q, C-16), 167.81 (s, C-1'), 119.89 (d, C-2'), 147.05 (d, C-3'), 15.57 (q, C-4') .
4. Gyimesi, J.; Melera, A. *Tetrahedron Lett.* **1967**, 1665-1673; Hanson, J. R.; Marten, T.; Siverns, M. *J. Chem. Soc. Perkin I* **1974**, 1033-1036.
5. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092-4096.
6. Data for **2**: HR-CIMS *m/z* 335.1859 (MH^+, calcd mass=335.1858); $[\alpha]_D^{19}$ -14.1 (c 0.1, MeOH); IR (neat) ν_{max} 3450, 1710 cm^{-1}; UV λ_{max}^{MeOH} 210 nm (ε=1.5 x 10^4); ^{1}H-NMR (300 MHz, $CDCl_3$), 2.58 (dd, *J* = 7.8, 15.5 Hz, H-3α), 2.04 (ddd, *J* = 3.6, 5.2, 15.5 Hz, H-3β), 4.15 (d, *J* = 5.8 Hz, H-8β); ^{13}C-NMR (75 MHz, $CDCl_3$), δ 36.91 (t, C-3), 67.83 (d, C-8).
7. Data for **3**: HR-CIMS *m/z* 351.1805 (MH^+, calcd mass=351.1807); $[\alpha]_D^{18}$ +28.8 (c 0.2, MeOH); IR ($CHCl_3$) ν_{max} 3420, 1705 cm^{-1}; UV λ_{max}^{MeOH} 211 nm (ε=1.2 x 10^4); ^{1}H-NMR (300 MHz, $CDCl_3$), δ 4.22 (ddd, *J* = 2.7, 3.0, 4.9 Hz, H-3β), 4.13 (d, *J* = 4.9 Hz, H-8β); ^{13}C-NMR (75 MHz, $CDCl_3$), δ 78.81 (d, C-3), 67.73 (d, C-8).
8. For a review, see: Murakami, A.; Ohigasi, H.; Koshimizu, K. *Biosci. Biotech. Biochem.* **1996**, *60*, 1-8.
9. Berenblum, I. *Cancer Res.* **1941**, *1*, 44-48; Pitot, H. C.; Dragan, Y. P. *FASEB J.* **1991**, *5*, 2280-2286.
10. Sporn, M. B. *Cancer Res.* **1976**, *36*, 821-830.

11. This study was financially supported in part by Grants-in-Aid from the Ministry of Education, Science, Sports, and Culture, and from the Ministry of Health and Welfare for the Second Term Comprehensive 10-Year Strategy for Cancer Control, Japan.

(*Received in Japan* 24 *September* 1996; *revised* 1 *November* 1996; *accepted* 5 *November* 1996)