BENADROSTIN, NEW INHIBITOR OF POLY(ADP-RIBOSE) SYNTHETASE, PRODUCED BY ACTINOMYGETES

II. STRUCTURE DETERMINATION

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Benadrostin, a new inhibitor of poly(ADP-ribose) synthetase, has been isolated from the culture broth of *Streptomyces flavovirens* MH499-O'F1. The structure of benadrostin was defined as 8-hydroxy-2*H*-1,3-benzoxazine-2,4-dione by an analysis of spectral properties and chemical studies of benadrostin and its derivatives.

In the preceding paper[1] we have described the taxonomy, the isolation, purification and the biological properties of benadrostin (**1**), a novel inhibitor of poly(ADP-ribose) synthetase. In this paper, we wish to describe the structure of **1**.

1 was obtained as colorless crystals. The molecular formula of **1** was elucidated as $C_8H_5NO_4$ by the high resolution (HR)-MS and elemental analysis.

The UV and IR spectra of **1** are shown in Figs. 1 and 2, respectively. The UV spectra showed maxima at 223 (log ε 4.30), 252 (sh, 3.74) and 307 nm (3.40) in MeOH, and 216 (log ε 4.65), 241 (4.43) and 327 nm (3.54) in 0.01 M NaOH - MeOH, indicating the presence of phenol moiety in the molecule. The IR spectrum (KBr) showed the absorption of the benzene ring (1620, 1590 and 1500 cm^{-1}), phe-

Fig. 1. UV spectrum of benadrostin (**1**).

—— Neutral λ_{max}^{MeOH} nm (log ε) 223 (4.30), 252 (sh, 3.74), 307 (3.40),
--- acidic $\lambda_{max}^{MeOH-HCl}$ nm (log ε) 223 (4.38), 252 (sh, 3.82), 308 (3.47),
——— basic $\lambda_{max}^{MeOH-NaOH}$ nm (log ε) 216 (4.65), 241 (4.43), 327 (3.54).

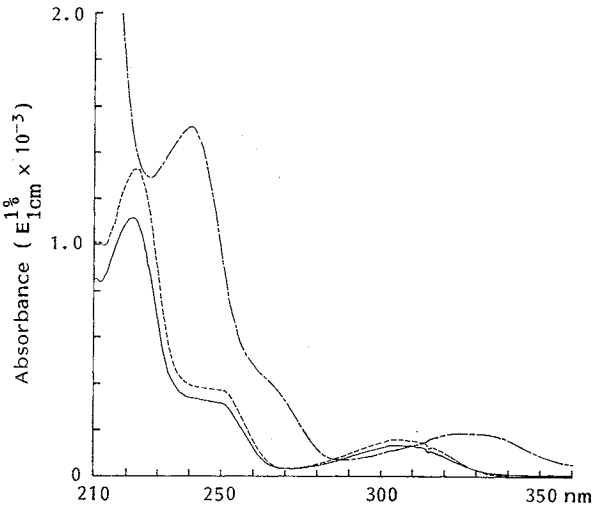


Fig. 2. IR spectrum of benadrostin (1).

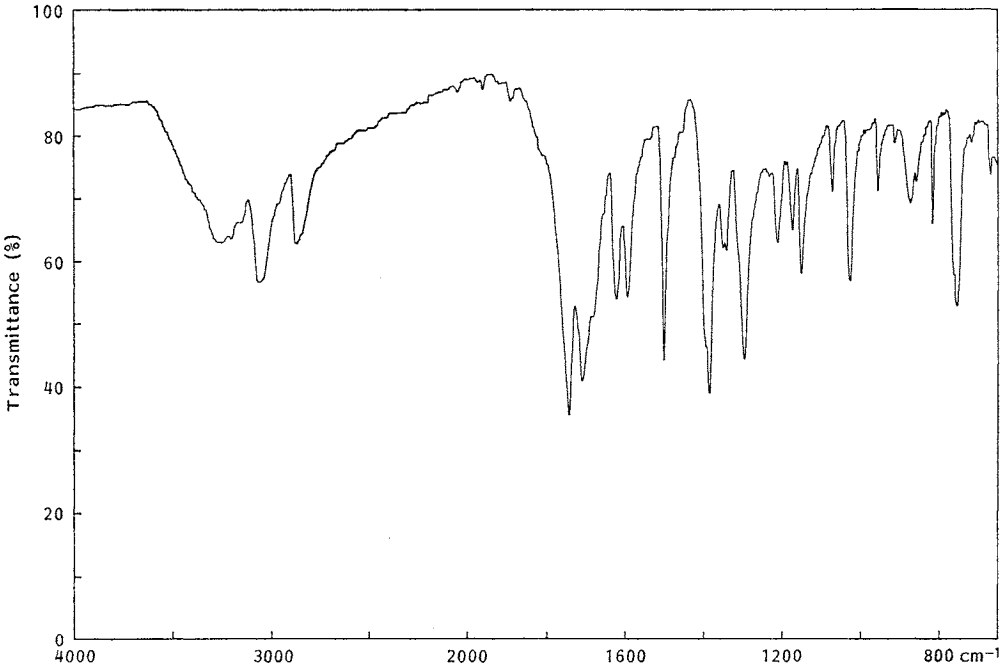


Table 1. ^{1}H NMR data[a] for benadrostin (1) and dimethylbenadrostin (2).

Proton	Benadrostin (1)	Dimethylbenadrostin (2)
3-H	10.67 (1H, br s)[b]	
5-H	7.48 (1H, dd, J=8.5, 1.5)	7.64 (1H, dd, J=8.3, 1.5)
6-H	7.25 (1H, t, J=8.5)	7.29 (1H, t, J=8.3)
7-H	7.32 (1H, dd, J=8.5, 1.5)	7.22 (1H, dd, J=8.3, 1.5)
8-OH	9.21 (1H, br s)[b]	
3-CH_3		3.48 (3H, s)
8-OCH_3		3.97 (3H, s)

[a] 400 MHz; chemical shifts in ppm, coupling constants in Hz.
[b] The assignments for these signals may be interchanged.
Benadrostin was measured in Me_2CO-d_6 and dimethylbenadrostin was in $CDCl_3$.

nolic hydroxyl (3260, 1380 and 1290 cm^{-1}), lactone (1740 cm^{-1}) and amide groups (1700 cm^{-1}).

For an elucidation of the structure, **1** was converted to a dimethyl derivative (**2**) by methylation with diazomethane. The molecular formula of **2** was determined as $C_{10}H_9NO_4$ by HR-MS and elemental analysis.

The ^{1}H and ^{13}C NMR data of **1** and **2** are presented in Tables 1 and 2. The ^{1}H and ^{13}C NMR spectra of **1** and **2** suggested the presence of 1, 2, 3 trisubstituted benzene ring. This was supported by alkaline hydrolysis of **1** to give 2,3-dihydroxybenzoic acid.

Two broad singlets corresponding to an amide and a hydroxyl group were observed at δ 10.67 and 9.21 in the ^{1}H NMR spectrum of **1**, while in that of **2** these peaks were absent and two methyl peaks appeared at δ 3.48 (NCH_3) and 3.97 (OCH_3).

The positional arrangement of the two carbonyl groups, one oxygen atom and one nitrogen atom

Table 2. ^{13}C NMR data[a] for benadrostin (**1**) and dimethylbenadrostin (**2**).

Carbon	Benadrostin (**1**)	Dimethyl-benadrostin (**2**)
C-2	147.6 (s)	147.9 (s)
C-4	161.9 (s)	160.8 (s)
C-4a	116.7 (s)	115.0 (s)
C-5	118.1 (d)	117.5 (d)
C-6	126.0 (d)	125.3 (d)
C-7	123.2 (d)	118.6 (d)
C-8	145.9 (s)	147.2 (s)
C-8a	143.7 (s)	142.5 (s)
3-CH_3		29.0 (q)
8-OCH_3		56.5 (q)

[a] 100 MHz; chemical shifts in ppm.
Benadrostin was measured in Me_2CO-d_6 and dimethylbenadrostin was in $CDCl_3$.

Fig. 3. LSPD and NOE experiments of dimethylbenadrostin (**2**).

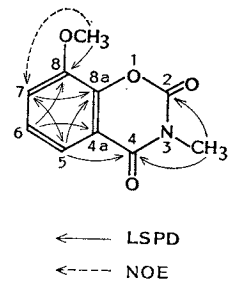
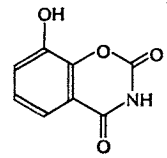


Fig. 4. Structure of benadrostin (**1**).



in the heterocyclic ring of **2** were defined by long range selective proton decoupling (LSPD) and nuclear Overhauser effect (NOE) experiments (Fig. 3). The methyl protons at δ 3.48 coupled with C-2 (δ 147.9) and C-4 (δ 160.8), demonstrating the arrangement from C-2 to C-4. A long range coupling between 5-H (δ 7.64) and C-4 (δ 160.8) indicated the bondage from C-4 to C-5. The methoxyl protons at δ 3.97 coupled with C-8 (δ 147.2), indicating its bonding position at C-8. A long range coupling between 6-H (δ 7.29) and C-8 clarified the arrangement from C-6 to C-8, which was also supported by the observation of an NOE between the 8-OCH_3 and 7-H (δ 7.22).

Thus, the structure of **1** was determined to be 8-hydroxy-2*H*-1,3-benzoxazine-2,4-dione (Fig. 4).

Experimental

UV spectra were recorded on a Beckman DU-8 spectrophotometer, and IR spectra on a Hitachi 260-10 spectrophotometer. Mass spectra [electron impact (EI)-MS, secondary ion (SI)-MS and HR-MS] were carried out on a Hitachi M-80H mass spectrometer. NMR spectra were recorded on a Jeol JNM-GX400 NMR spectrometer with ^1H NMR at 400 MHz and ^{13}C NMR at 100 MHz. MP's were measured with a micro melting point apparatus MP-S3 (Yanagimoto Seisakusyo Co., Japan) and were uncorrected.

Methylation of **1**

A solution of diazomethane in diethyl ether was added to a solution of 10 mg of **1** in 1 ml of MeOH, and the mixture was allowed to stand for 1 hour at room temp. Excess diazomethane and solvents were removed from the mixture under reduced pressure. The product was subjected to silica gel TLC with $CHCl_3$ - MeOH (10:1). The extract from the Rf 0.78 fraction was concentrated to dryness. It was dissolved in 2 ml of MeOH and applied to Sephadex LH-20 eluting with MeOH to give **2** as colorless powder. **2**: Rf 0.68 ($CHCl_3$ - MeOH, 10:1); mp 122~124°C; MS *m/z* 207 (M, $C_{10}H_9NO_4$); UV λ_{max}^{MeOH} nm (log ε) 223 (4.36), 250 (sh, 3.75), 303 (3.36); IR (KBr) cm^{-1} 2950, 1750, 1690, 1620, 1600, 1500, 1420, 1360, 1300, 1120, 1080, 750. The ^1H and ^{13}C NMR data are presented in Tables 1 and 2, respectively.

Anal Calcd for $C_{10}H_9NO_4$: C 57.97, H 4.38, N 6.76.
Found: C 57.83, H 4.47, N 6.47.

Hydrolysis of **1**

NaOH solution (2 N, 120 μl) was added to a solution of **1** (10 mg) in MeOH and heated for 2 hours at 75°C in a sealed tube. The solution was adjusted to pH 2.0 with 1 N HCl, and extracted with an equal volume of EtOAc. The solution in EtOAc was subjected to silica gel TLC with $CHCl_3$ - MeOH (1 : 1). The extract from the Rf 0.69 fraction was concentrated to dryness. It was dissolved in 2 ml of MeOH and applied to a Sephadex LH-20 column eluted with MeOH to give 2,3-dihydroxybenzoic acid as a colorless powder. Rf 0.69 ($CHCl_3$ - MeOH, 1 : 1); mp 200~202°C (mp 203~205°C)[2]; MS *m/z* 154 (M, $C_7H_6O_4$), 136 (M$-H_2O$); UV λ_{max}^{MeOH} nm (log ε) 246 (3.76), 315 (3.49), [245 (3.78), 314 (3.48)][3]; IR (KBr) cm^{-1} 3400, 3100, 1680, 1610, 1480, 1360, 1300, 1260, 1170, 1080, 840, 750; ^1H NMR (400 MHz, CD_3OD) δ 6.55 (1H, t, J=8.0 Hz), 6.83 (1H, dd, J=8.0 and 1.8 Hz), 7.33 (1H, dd, J=8.0 and 1.8 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 118.0, 118.7, 120.5, 122.4, 146.2, 151.3, 176.2.

Anal Calcd for $C_7H_6O_4$: C 54.55, H 3.92.
Found: C 54.03, H 3.86.

References

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