BE-23372M, A NOVEL PROTEIN TYROSINE KINASE INHIBITOR

II. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE ELUCIDATION

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BE-23372M, a new protein tyrosine kinase inhibitor, has been obtained as a reddish orange solid. The compound, $C_{17}H_{12}O_6$, HRFAB-MS: m/z 312.0625 $(M)^+$, is an acidic substance, showing UV (MeOH) λ_{max} (ε) 266 (8,800), 426 nm (20,400), and IR (KBr) ν_{max} 1752(C=O) and 3298(OH) cm^{-1}. The structure of BE-23372M, (*E*)-3-(3,4-dihydroxybenzylidene)-5-(3,4-dihydroxyphenyl)-2(3*H*)-furanone, has been elucidated by ^1H and ^{13}C NMR studies.

In the preceding paper, the taxonomy and fermentation of the producing organism, as well as the isolation and biological activities of BE-23372M have been described[1]. We report here the physico-chemical properties and the structure elucidation of BE-23372M, a new protein tyrosine kinase inhibitor.

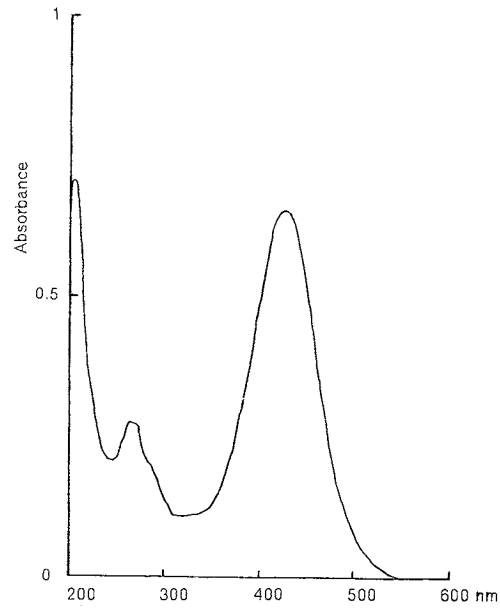
Physico-chemical Properties

BE-23372M was obtained as a reddish orange solid. The compound was acidic and soluble in dimethyl sulfoxide, acetone and methanol, hardly soluble in chloroform or ethyl acetate, insoluble in *n*-hexane. It gave Rf value of 0.27 (chloroform-methanol-formic acid, 20:2:1) on TLC (Kieselgel 60 F_{254}, Merck). The substance showed positive color reactions with $KMnO_4$ and $FeCl_3$.

The HRFAB-MS revealed the molecular ion at m/z 312.0625 (M^+) which was consistent with the molecular formula of $C_{17}H_{12}O_6$ (calcd 312.0634). The UV spectrum showed maxima at 266 (ε 8,800) and 426 nm (ε 20,400) in methanol (Fig. 1). The IR spectrum (KBr) indicated the presence of γ-lactone (1752 cm^{-1}) and phenolic hydroxy groups (3298 cm^{-1}) (Fig. 2).

The ^{13}C and ^1H NMR data in $(CD_3)_2CO$ measured at 100 MHz and 300 MHz, respectively, are presented in Table 1.

Fig. 1. The UV spectrum of BE-23372M in MeOH.



Structure Elucidation

The result of HRFAB-MS gave the molecular formula of $C_{17}H_{12}O_6$ for BE-23372M, which was supported by ^1H and ^{13}C NMR spectra.

The ^{13}C NMR spectrum of BE-23372M in-

Fig. 2. The IR spectrum of BE-23372M in KBr.

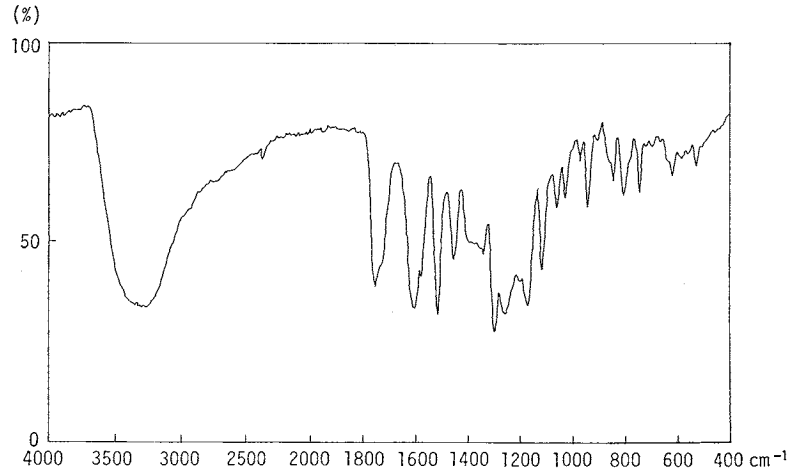


Table 1. ^{13}C NMR and ^{1}H NMR data of BE-23372M.

Carbon	Chemical shift (ppm)	
	δ_C	δ_H
1	170.2	
2	123.3	
3	99.0	7.10 (1H, s)
4	156.7	
5	121.5	
6	112.9	7.32 (1H, d, J=2.1 Hz)
7	146.3	
8	148.5	
9	116.5	6.93 (1H, d, J=8.3 Hz)
10	118.8	7.26 (1H, dd, J=8.3, 2.1 Hz)
11	134.5	7.15 (1H, s)
12	128.6	
13	117.5	7.33 (1H, d, J=2.1 Hz)
14	146.3	
15	148.7	
16	116.7	6.94 (1H, d, J=8.3 Hz)
17	124.9	7.24 (1H, dd, J=8.3, 2.1 Hz)
OH		8.5 (4H, br s)

Fig. 3. Possible structures of conjugated butenolides.

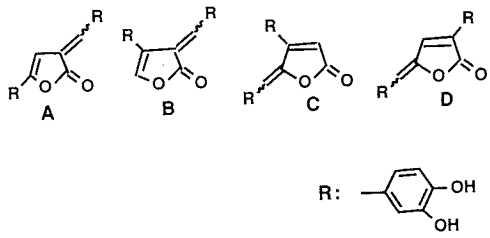
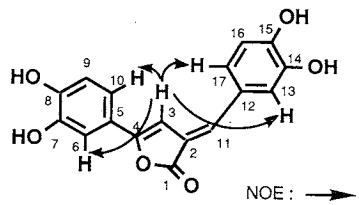


Fig. 4. The structure of BE-23372M.



dicated the presence of one carbonyl carbon and 16 aromatic or olefinic carbons. In the ^{1}H NMR spectrum, the analysis of proton coupling revealed the existence of two 4-substituted catechol rings[2]. The IR spectrum of BE-23372M suggested the presence of γ-lactone (1752 cm^{-1}).

Since 12 aromatic carbons of BE-23372M were attributed to catechol rings, 4 olefinic carbons and one carbonyl carbon should belong to a conjugated butenolide. As two olefinic protons (3-H and 11-H; δ_H 7.10 and 7.15, respectively) were singlets, 4 possible conjugated butenolides (**A**~**D**) were proposed (Fig. 3).

The HMBC spectrum of BE-23372M revealed that the two olefinic protons (3-H and 11-H) were coupled to the carbonyl carbon (C-1; δ_C 170.2) and that two aromatic protons (6-H and 10-H; δ_H 7.32

and 7.26, respectively) were coupled to an enolic carbon (C-4; δ_C 156.7). These data supported the structure **A**. Assignments of signals in ^{13}C and ^{1}H NMR spectra were made by C-H COSY and HMBC.

Finally, the configuration of the olefin was determined by NOE experiment. As shown in Fig. 4, NOE was observed between 3-H (δ_H 7.10) and 4 aromatic protons (6-H, 10-H, 13-H and 17-H; δ_H 7.32, 7.26, 7.33 and 7.24, respectively). This result indicated that the configuration was *E*.

Thus, the structure of BE-23372M was elucidated to be (*E*)-3-(3,4-dihydroxybenzylidene)-5-(3,4-dihydroxyphenyl)-2(3*H*)-furanone (Fig. 4).

Among natural products, aspulvinones[3,4] are somewhat similar to BE-23372M. However, the structures of the conjugated butenolides are considerably different. Thus, the biosynthesis of BE-23372M is another interest.

References

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