Communications to the Editor

ADECYPENOL, A UNIQUE ADENOSINE DEAMINASE INHIBITOR CONTAINING HOMOPURINE AND CYCLOPENTENE RINGS

Sir:

In the course of our screening for enzyme inhibitors from microorganisms, a new adenosine deaminase inhibitor, which was named adecypenol, was isolated from the cultured broth of *Streptomyces* sp. OM-3223. The structure was determined based on spectral data. It has the homopurine aglycone, identical with that of the known adenosine deaminase inhibitors coformycin, 2′-deoxycoformycin and adechlorin, which attaches to the C-3 position of 5-(hydroxymethyl)-4-cyclopentene-1,2-diol found in neplanocin A. This paper describes the isolation, physico-chemical properties and structure determination of adecypenol.

The producing strain was isolated from a soil sample collected at Inokashira Park, Musashino-City, Tokyo, Japan. The cultured broth (20 liters) of the strain was centrifuged to obtain about 18 liters of a supernatant fluid, which was applied to a column of activated carbon. After the column was washed with H_2O, the active material was eluted with 7.0% aqueous Me_2CO. The active eluate was concentrated *in vacuo* and freeze-dried to give a brown powder. It was dissolved in a small volume of H_2O and poured into EtOH. After the removal of the flocculated material by filtration, the filtrate was evaporated to dryness. The resulting brown paste was chromatographed on a Diaion HP-20 column with 10.0% aqueous MeOH. The active fractions were combined, concentrated under the reduced pressure and freeze-dried to yield a crude powder (4.1 g). The powder was purified on a Toyopearl HW-40 column with EtOH - H_2O (1: 9). The active fractions were combined and then freeze-dried to give a pale brown powder (600 mg). The powder was chromatographed on reversed phase silica gel using H_2O - MeOH. The fractions containing the active principles were collected separately and then evaporated *in vacuo* to yield a yellow powder (120 mg) and a white powder (100 mg), respectively. Further purification was carried out by a preparative HPLC column of reversed phase silica gel (ODS) with 5.0% aqueous MeOH as developing solvent. From the yellow and white powders, pure samples of adecypenol (19 mg) and another inhibitor (20 mg) were obtained as white amorphous powders, respectively. The latter was identified with coformycin by ^1H NMR analysis.

Adecypenol is readily soluble in H_2O and MeOH, but practically insoluble in EtOAc, $CHCl_3$ and Me_2CO. It gave positive reactions with H_2SO_4 and $KMnO_4$, but negative to ninhydrin. Adecypenol was crystallized from H_2O to give colorless needles, mp 240~245°C (dec), $[\alpha]_D^{24}$ −31.6° (*c* 1.0, H_2O). The UV [$\lambda_{max}^{H_2O}$ nm (ε) 279 (7,960)] and IR [ν_{max}^{KBr} cm^{-1} 3400, 1640, 1380, 1200, 1120] data of adecypenol closely resembled those of adechlorin[1], coformycin[2] 2′-deoxycoformycin[3]. The molecular formula was established to be $C_{12}H_{16}N_4O_4$ by high-resolution mass spectroscopy [calcd 280.1170 (M^+), found 280.1174]. It was also supported by elemental analysis [calcd C 51.42, H 5.75, N 19.99. Found C 50.77, H 5.65, N 19.87].

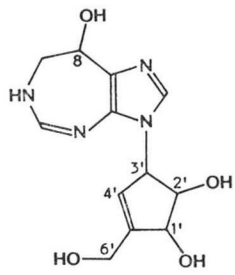
The ^1H NMR data in D_2O suggested the existence of the same aglycone with adechlorin[1], coformycin[4] and 2′-deoxycoformycin[3], which have a 3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]-diazepin-8-ol moiety, *i.e.*, 3.31 (d, 1H), 3.42 (dd, 1H), 5.06 (br d, 1H), 7.10 (s, 1H) and 7.35 (br s, 1H) ppm. The fragment ion peak at *m/z* 152 [$C_6H_8N_4O$ (chromophore+1); HI-MS calcd 152.0698, found 152.0699] of EI-MS also indicated the presence of above-mentioned chromophore. The ^{13}C NMR data of adecypenol confirmed that the chromophore [47.4 (t), 67.2 (d), 128.4 (s), 131.5 (d), 136.1 (s), 150.1 (d) ppm] was identical with those of adechlorin, coformycin and 2′-deoxycoformycin. From the findings that the treatment with acetic anhydride and pyridine give pentaacetate [*m/z* 490 (M^+)], and that the remained formula is $C_6H_9O_3$, the existence of three hydroxyl groups in the portion was indicated.

The remained structure was determined by ^1H NMR decoupling experiments at 400 MHz as summarized in Table 1. The proton signal at 4.12 ppm (H-2′) coupled with the signals at 4.55

Table 1. ^{1}H NMR data of cyclopentene system of adecypenol in D_2O.

Protons	Chemical shifts (ppm)	Coupling constans (Hz)			
H-1′	4.55	1′, 2′; 5.6	1′, 3′; 1.0	1′, 6′; <1.0	
H-2′	4.12	2′, 1′; 5.6	2′, 3′; 5.4		
H-3′	5.26	3′, 1′; 1.0	3′, 2′; 5.4	3′, 4′; 2.0	3′, 6′; 2.2
H-4′	5.83	4′, 3′; 2.0	4′, 6′; 1.6		
H-6′	4.23	6′, 1′; <1.0	6′, 3′; 2.2	6′, 4′; 1.6	

Fig. 1. Structure of adecypenol.



(H-1′) and 5.26 (H-3′) ppm. The latter proton also coupled with the signal at 5.83 (H-4′) ppm. The chemical shift of this proton attributed to be that of olefinic proton or anomeric proton. Since the ^{13}C NMR spectrum displayed the presence of one protonated sp^2 carbon in this portion [58.9 (t), 63.1 (d), 73.1 (d), 78.5 (d), 126.4 (d), 147.9 (s) ppm], the proton at 5.83 ppm should be the olefinic proton. It also coupled with oxygenated methylene proton at 4.23 (H-6′) ppm with allyl coupling. Because the coupling between the signals at 4.55 and 5.83 ppm could not be observed, the structure of remained portion was concluded to be 5-(hydroxymethyl)-1,2,3-trisubstituted-4-cyclopentene. The chemical shift (5.26 ppm) of proton at C-3′ confirmed that the chromophore connected at this position. This cyclopentene system have been reported in the structure of neplanosin A and the published ^{1}H NMR data of this system[5] coincide with those of adecypenol. Thus, the structure of adecypenol was determined as shown in Fig. 1. Although the stereochemistry is under investigation, that of carbon 8 should be *R* configuration because 8-*R* configuration of 2′-deoxycoformycin has been reported to be essential for adenosine deaminase inhibitor[6].

The details of the taxonomy and fermentation of the producing strain and the biological activities of adecypenol will be published in the near future.

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