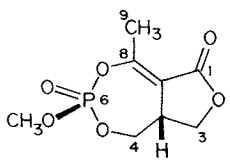
CYCLOPHOSTIN,† ACETYLCHOLINESTERASE INHIBITOR FROM *Streptomyces lavendulae*

Sir:

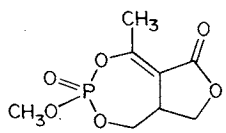
Many synthetic organophosphates have been known as insecticides for a long time. Recently, natural cyclic organophosphates were first isolated as natural insecticides and acetylcholinesterase inhibitors from *Streptomyces antibioticus* strain DSM 1951.[1] In the course of our screening program for natural insecticides of microbial origin, we isolated a new product, cyclophostin (**1**), from *Streptomyces lavendulae* strain NK901093 as a strong inhibitor of acetylcholinesterase. Cyclophostin (**1**) showed one of the strongest inhibitory activity values for the acetylcholinesterase of houseflies: I_{50} 7.6×10^{-10} M. We report here the isolation and structure of compound **1** including its absolute stereochemistry. Cyclophostin (**1**) is probably the same as TAN-1139, a compound disclosed in the Japanese patent literature but whose structure has not been previously described.[2]

Cyclophostin (**1**) was isolated as crystals from the fermentation broth of *Streptomyces lavendulae*: MP 113~114°C (*n*-hexane-EtOAc); $[\alpha]_D^{20}$ −7.56° (*c* 1.35, EtOH). The molecular formula $C_8H_{11}O_6P$ was determined by HREI-MS, ^{13}C, ^{31}P, and ^1H NMR, and elemental analysis. ^1H, ^{13}C, and ^{31}P NMR data indicated the presence of olefinic CH_3, OCH_3, and phosphate groups. As shown in Table 1, the ^1H NMR signals of two 4-H protons and 6-OCH_3 protons split into doublets due to the coupling with ^{31}P atom at the 6-position, in addition to the normal ^1H-^1H coupling. The ^1H NMR signal for the 8-CH_3 protons splits into a doublet due to the long range homoallyl spin coupling with 3a-H; when the 3a-H was irradiated, the 8-CH_3 doublet signal became a singlet, and in the ^1H-^1H COSY spectrum, a cross peak was observed between 8-CH_3 and 3a-H. Most of the ^{13}C signals of **1** also split into doublets, in addition to the splits due to the normal ^1H-^{13}C coupling, indicating long range ^{13}C-^{31}P coupling (Table 2). From the ^1H-^1H and ^1H-^{13}C COSY data, the partial structure of $-OCH_2-CH-CH_2O-$ was obtained (Tables 1 and 2). The two intense bands in the IR spectrum at 1759 and 1672 cm^{-1} indicated the presence of γ-lactone and enol ether moieties, respectively. The UV spectrum also indicated the presence of a conjugated lactone: λ_{max} 225.8 nm (ε 10,700). On the basis of these spectroscopic data, the possible plane structure **1a** was deduced. To confirm this structure and to determine the relative and absolute stereochemistry of (−)-**1**, we next performed the X-ray crystallographic structure analysis.

Compound (−)-**1** was recrystallized from diethyl ether-ethyl acetate to give clear prisms, which were found to be monoclinic and the space group to be $P2_1$. As illustrated in the ORTEP drawing of Fig. 1, cyclophostin (**1**) has a cyclic phosphate moiety and a molecular framework of a five-seven membered ring system. The absolute stereochemistry of (−)-**1** was determined to be (3a*R*, 6*S*) by the anomalous scattering method (Table 3). Related compounds with a similar molecular



(3a*R*,6*S*)-(−)-**1**



1a

Table 1. ^1H NMR and ^1H-^1H COSY NMR data of cyclophostin (**1**) in $CDCl_3$.[a]

Position	^1H NMR	^1H-^1H COSY
3	δ 3.747 (1H, m) 4.412 (1H, m)	4.412 (3-H)
3a	3.76~3.80 (1H, m)	4.135 (4-H), 4.301 (4-H), 4.412 (3-H)
4	4.135 (1H, ddd, J=25.5, 11.1, 3.6 Hz), 4.301 (1H, ddd, J=11.1, 10.9, 6.2 Hz)	4.301 (4-H)
6-OCH_3	3.901 (3H, d, J=11.5 Hz)	—
8-CH_3	2.418 (3H, d, J=2.0 Hz)	3.76~3.80 (3a-H)

[a] ^1H NMR (399.7 MHz: in ppm (δ) downfield from tetramethylsilane).

† Cyclophostin was originally called as NK901093.

Table 2. ^{13}C NMR and ^{1}H-^{13}C COSY NMR data of cyclophostin (**1**) in $CDCl_3$.[a]

Position	^{13}C NMR	^{1}H-^{13}C COSY
1	δ 168.76 (d, J=1.8 Hz)	—
3	64.10 (td, J=153.7, 4.0 Hz)	3.747 (3-H), 4.412 (3-H)
3a	39.53 (d, J=137.9 Hz)	3.76~3.80 (3a-H)
4	67.43 (td, J=150.5, 6.0 Hz)	4.135 (4-H), 4.301 (4-H)
8	161.25 (d, J=7.3 Hz)	—
8a	111.96 (d, J=3.7 Hz)	—
6-OCH_3	55.76 (qd, J=149.8, 6.4 Hz)	3.901 (6-OCH_3)
8-CH_3	18.00 (qd, J=130.5, 4.4 Hz)	2.418 (8-CH_3)

[a] ^{1}H NMR (100.4 MHz: in ppm (δ) downfield from tetramethylsilane).

Table 3. Observed and calculated structure factor relations between some (hkl) and (−h−k−l) reflections of cyclophostin (3a*R*,6*S*)-(−)-(**1**).[a]

h	k	l	$\lvert F_o(hkl)\rvert$ [$\lvert F_c(hkl)\rvert$]	$\lvert F_o(-h-k-l)\rvert$ [$\lvert F_c(-h-k-l)\rvert$]	$\lvert F_o(hkl)\rvert/\lvert F_o(-h-k-l)\rvert$ [$\lvert F_c(hkl)\rvert/\lvert F_c(-h-k-l)\rvert$]
3	1	−5	49.1 [44.7]	40.8 [37.5]	1.20 [1.19]
3	1	−3	18.5 [15.7]	24.1 [19.9]	0.77 [0.79]
4	1	−3	42.6 [41.3]	44.8 [45.0]	0.95 [0.92]
2	2	−1	26.5 [26.0]	32.5 [32.0]	0.81 [0.81]
2	3	0	30.1 [28.0]	23.8 [21.9]	1.26 [1.28]
3	2	0	39.7 [33.1]	45.3 [41.2]	0.88 [0.80]
4	3	0	22.3 [17.4]	16.7 [11.6]	1.34 [1.50]
1	2	1	55.8 [49.6]	47.7 [42.5]	1.17 [1.17]
2	1	1	26.7 [25.2]	21.7 [21.2]	1.22 [1.19]
3	1	1	37.7 [34.0]	28.3 [25.3]	1.33 [1.34]
3	3	1	54.8 [48.0]	45.2 [41.3]	1.21 [1.16]
5	2	1	16.5 [16.1]	21.7 [22.1]	0.76 [0.73]
4	3	2	26.9 [21.7]	20.0 [16.2]	1.34 [1.34]
0	2	4	48.6 [44.9]	54.0 [51.4]	0.90 [0.87]
2	1	4	43.1 [40.6]	46.8 [45.3]	0.92 [0.90]
3	2	4	50.2 [45.8]	52.8 [50.9]	0.95 [0.90]

[a] Reflections which satisfy $\|\lvert F_o(hkl)\rvert - \lvert F_o(-h-k-l)\rvert\| > 10\sigma(F_o)$ were selected, where $\sigma(F_o) = [\sigma_{count}{}^2 + (0.007\lvert F_o\rvert)^2]^{0.5}$.

framework were previously isolated as natural insecticides and acetylcholinesterase inhibitors from *Streptomyces antibioticus* strain DSM 1951.[1)]

The inhibitory activities of cyclophostin (**1**) against acetylcholinesterase (AChE) are shown in Table 4. AChE inhibitory activities were measured by the modified method of ELLMAN *et al.*[3)] The potency of cyclophostin (**1**) as an AChE inhibitor was higher than other two compounds in both the cases of the housefly and brown planthopper; the activity of cyclophostin (**1**) against the AChE of housefly, CSMA strain, is 8,000 and 60 times stronger than those of propaphos and physostigmine sulfate, respectively. Similarly, in the case of the AChE of brown planthopper, Kaseda strain, cyclophostin (**1**) is 70,000 and 70 times more active than propaphos and physostigmine sulfate, respectively.

Table 4. Inhibitory activities of cyclophostin (**1**) and some compounds against acetylcholinesterase[a] from housefly; CSMA strain and from brown planthopper; Kaseda strain.

Compounds	AChE I_{50}-value (M)	
	Housefly	Brown planthopper
Cyclophostin	7.6×10^{-10}	1.3×10^{-9}
Propaphos	6.2×10^{-6}	9.6×10^{-5}
Physostigmine sulfate	5.0×10^{-8}	9.1×10^{-8}

[a] Eighty heads of female houseflies *Musca domestica* and brown planthoppers *Nilaparvata lugens* were homogenized with 10 ml of 1/15 M phosphate buffer (pH 7.4). The homogenates were centrifuged at 2,000 rpm for 20 minutes at 0°C. The supernatants were used as the enzyme solution.

Isolation of Cyclophostin (3a*R*, 6*S*)-(−)-(**1**)

A loopful of the slant culture of *Streptomyces lavendulae* strain NK901093 was inoculated to 500-ml Erlenmeyer flasks containing 100 ml of the seed medium composed of glycerin 2.0%, soybean meal 2.0%, NaCl 0.3% (pH 7.0). The flasks were shaken on a rotary shaker (220 rpm) at 27°C for 2 days. The growth (2 ml) was transferred to every 500-ml Erlenmeyer flasks containing 100 ml of the same medium as described above. The fermentation was carried out on a rotary shaker (220 rpm) at 27°C for 4 days.

The fermentation broth was filtered, and the filtrate (9 liters, pH 5.6) was applied on a column of Diaion HP-20 (500 ml) and eluted with 50% aqueous acetone (1 liter).

The aqueous acetone solution was extracted with ethyl acetate (1 liter) three times. The ethyl acetate layer was evaporated to dryness *in vacuo* to give a brown syrup (1.43 g). The active syrup was applied on a column of silica gel (50 g, Wako gel C-200) and was eluted with a mixture of dichloromethane - ethyl acetate (9 : 1).

The active fractions were evaporated to dryness *in vacuo* to give a colorless powder (141 mg). The powder was recrystallized from *n*-hexane - ethyl acetate to give colorless crystals (76 mg) of cyclophostin (**1**): MP 113~114°C; IR (KBr) ν_{max} 3426, 2919, 1759, 1672, 1480, 1428, 1288, 1259, 1239, 1212, 1116, 1080, 1042, 1024, 1000, 837, 766, 706 cm^{-1}; ^{31}P NMR (36.28 MHz, D_2O, in ppm (δ) downfield from phosphoric acid as an internal standard) δ −7.6 (m); $[\alpha]_D^{20}$ −7.56° (*c* 1.35, EtOH); UV (EtOH) λ_{max} 225.8 nm (ε 10,700). High resolution mass spectrum (HRMS), calcd for $C_8H_{11}O_6P$: 234.0293. Found: 234.0295. *Anal.* Calcd for $C_8H_{11}O_6P$: C 41.04, H 4.74. Found: C 41.24, H 4.71.

X-Ray Crystallography of Cyclophostin (−)-(**1**)

Single crystals were obtained as colorless prisms by crystallization of cyclophostin (**1**) from diethyl ether - ethyl acetate. A crystal (dimension 0.23 × 0.30 × 0.45 mm) was selected for data collection and mounted on a Rigaku AFC-6B automated four circle diffractometer. The crystal was found to be monoclinic, and the unit cell parameters and orientation matrix were obtained. Data collection was carried out by use of a 2θ-θ scan: formula, $C_8H_{11}O_6P$; formula weight, 234.15; space group, $P2_1$; a = 9.821(1) Å, b = 5.988(0.6) Å, c = 8.681(1) Å, β = 103.14(1) degree; vol = 497.11 (9) $Å^3$; Z = 2; ρ(calcd) = 1.560 g/cm^3; ρ(obsd) = 1.554 g/cm^3 (by flotation using a CCl_4 - hexane solution); radiation, CuKα (1.541 78 Å); monochromator, graphite crystal; linear absorption coefficient, 24.38 cm^{-1}; temperature, 20°C; scan type, 2θ-θ; scan speed, 2.0°/minute; scan range, 1.5° + 0.3° tan θ; 2θ scan limits, 2° ~ 130°; standard reflections, 3 per 50 reflections; indices, (3, 0, 0), (0, 2, 0), (1, 0, 3); crystal stability, no indication of standard reflection decay during data collection; total reflections scanned, 1044; unique data $F_o > 3.0\sigma$ (F_o), 866. To determine the absolute configuration of the structure, 970(−h −k −l) reflections were also measured.

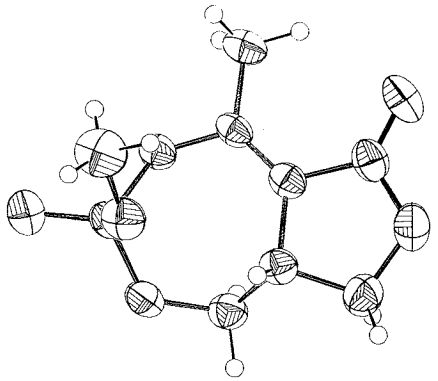


Fig. 1. ORTEP drawing of cyclophostin (3a*R*,6*S*)-(−)-(**1**). The atoms are drawn as 50% probability ellipsoids.

The position of the phosphorus atom was at first found by the direct method, and then those of the remaining non-hydrogen atoms were found by the successive Fourier syntheses. All hydrogen atoms were found by the difference Fourier syntheses. Absorption correction was made by use of the statistical method developed by C. KATAYAMA *et al.*[4] Full matrix least-squares refinement of positional parameters, anisotropic thermal parameters for non-hydrogen atoms, and parameters for the absorption correction, including anomalous scattering factors of phosphorus, oxygen, and carbon atoms, led to the final convergence with $R = 0.0266$ and $R_w = 0.0325$ (final no. of variables, 178) for the (3a*R*, 6*S*) absolute configuration,† while a similar calculation for the mirror image structure

† The isotropic thermal parameters of hydrogen atoms were not included as variables in the refinement, but calculated from the thermal parameters of the non-hydrogen atoms to which the hydrogen atoms were attached. The atomic parameters, bond lengths and bond angles have been sent to the Cambridge Crystallographic Data Centre. The list of observed and calculated structure factors may be obtained from one of the authors (N.H.) upon request.

gave $R=0.0293$ and $R_w=0.0360$; without the absorption correction, $R=0.0350$ and $R_w=0.0407$ for (3a*R*, 6*S*)-**1**, while $R=0.0373$ and $R_w=0.0435$ for the mirror image. In addition, the (3a*R*, 6*S*) absolute configuration of **1** was also determined by the Bijvoet method. The observed and calculated structure factors for some Bijvoet pairs listed in Table 3 along with their relative intensity ratios indicate that the absolute stereochemistry of cyclophostin (−)-(**1**) is (3a*R*, 6*S*) as illustrated in Fig. 1.

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