CHEMISTRY LETTERS, pp. 2375-2378 1992.

Cyclamenoside, a Potent Inhibitor of Hydrogen Peroxide Release

Naomi SAKAI, Chieko FUKUI, and Yoshikazu SHIZURI*
Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770

Cyclamenoside, a glucoside ester of gallic acid was isolated from *Cyclamen persicum* Miller as a potent inhibitor of hydrogen peroxide release from peritoneal neutrophils of rat. The structure of the active compound was determined by some chemical reactions and mainly by using ^{1}H and ^{13}C NMR spectroscopy.

It is well known that many natural phenolic compounds have wide ranges of biological and pharmacological activities. [1] Many of them have potent antioxidant activities such as scavenging hydrogen peroxide, superoxide anion, hydroxy radical and peroxyradical or quenching singlet oxygen, thus inhibiting lipid-peroxidation in the biological system *in vitro*. Therefore, these compounds are promising therapeutic drugs to maintain human health and to delay the aging processes by the protection of membranes from abnormal lipid-peroxidation. Thus, much effort has been made in our laboratory to search for natural antioxidants from the water soluble parts of plant extracts, resulting in the isolation of a potent inhibitor of hydrogen peroxide production from peritoneal neutrophils. We report herein the isolation, structure determination and biological activities of cyclamenoside (**1**) from *Cyclamen persicum* Miller.

Methanol extract of stems (180 g) of *C. persicum* was partitioned with ethyl acetate and Water. The water layer was further partitioned with butanol and water. The butanol layer was chromatographed on silica gel eluting with acetone-methanol-water (10 : 1 : 0.2). A red fraction was further chromatographed on silica gel with acetone-methanol-water (10 : 1 : 0.2) to afford a fraction containing anthocyanin, flavonoides and cyclamenoside, which was separated by reversed phase HPLC with methanol-water (1 : 1) to afford crude cyclamenoside. Pure cyclamenoside (**1**) [3] (9 mg, see Fig. 1) was obtained by reversed phase HPLC with methanol-water (3 : 7).

On acetylation, tetradecaacetate (**2**) [4] was obtained in quantitative yield. The ^{1}H and ^{13}C NMR signals were assigned by H-H and C-H COSY spectra of both compounds (**1** and **2**). From the spectral data, cyclamenoside has been shown to contain three moles of gallic acid and two moles of glucose (see Table 1 and 2).[5] Two signals of aromatic protons of the gallic acid unit (**B**) in **1** were observed separately at δ 7.47 and 7.36 as doublets, indicating that the gallic acid unit (**B**) was unsymmetrically connected with the other groups (see Table 1). And the signals of the protons of remaining two gallic acid units (**A** and **C**) were observed as two singlets at δ 7.11 and 7.07, showing that they were symmetrical (see Table 1). From the ^{1}H NMR spectra of the acetate(**2**) measured in $CDCl_3$ and C_6D_6, the structural feature mentioned above was also evident.

Furthermore, the connection of three gallic acid parts and two glucose units were proved by using

Table 1. Signals of aromatic protons

	Gallic acid A	Gallic acid B		Gallic acid C
Position	2,6	2	6	2,6
1 (CD_3OD)	7.07 or 7.11 (2H, s)	7.47 or 7.36 (1H, d, J=2.0 Hz)	7.36 or 7.47 (1H, d, J=2.0 Hz)	711 or 7.07 (2H, s)
2 ($CDCl_3$)	7.80 or 7.81 (2H, s)	7.64 (1H, d, J=1.8 Hz)	7.55 (1H, d, J=1.8 Hz)	7.81 or 7.80 (2H, s)
2 (C_6D_6)	8.07 or 8.11 (2H, s)	7.82 (1H, d, J=1.8 Hz)	7.80 (1H, d, J=1.8 Hz)	8.11 or 8.07 (2H, s)

Table 2. Signals of protons of the sugar parts

	Glucose **A**							Glucose **B**						
Position	1	2	3	4	5	6	6'	1	2	3	4	5	6	6'
1(CD_3OD)	5.64,d	≈3.5	≈3.5	≈3.5	≈3.77	a)	b)	≈4.86	≈3.5	≈3.5	≈3.5	≈3.77	a)	b)
Hz	8.1													
2 ($CDCl_3$)	5.22,d	5.57,dd	5.34,t	5.23,t	4.06,m	4.41,dd	4.48,dd	5.74,d	5.27,dd	5.40,t	5.21,t	4.30,m	4.46,dd	4.66,dd
Hz	7.6	7.6,9.5	9.5	9.5		3.7,12.2	2.0,12.2	8.3	8.3,9.5	9.5	9.5		3.7,12.2	4.4,12.2
2 (C_6D_6)	5.16,d	5.57,dd	5.64,t	5.22,t	4.23,m	4.40,m	4.58,dd	5.90,d	5.62,dd	5.67,t	5.48,t	3.85,m	4.40,s	4.40,s
Hz	7.9	7.9,9.7	9.7	9.7			5.9,12.0	8.2	8.2,9.7	9.7	9.7			

a) 4.41 or 4.42 (each 1H, dd, J=5.1, 12.5 Hz). b) 4.55 or 4.62 (each 1H, dd, J=1.7, 12.5 Hz).

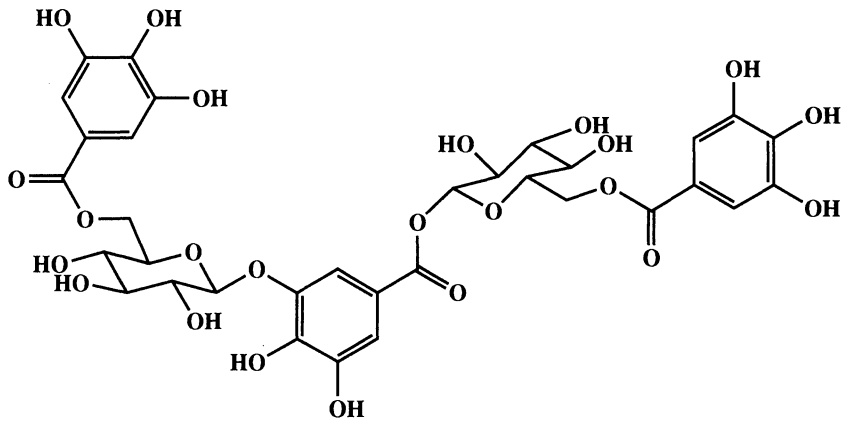

Fig. 1. The structure of cyclamenoside (**1**).

HMBC technique as mentioned below (see Fig. 2). Each methylene protons of C-6 positions of glucose A (δ 4.40 and 4.58) and B (δ 4.40) showed correlation peaks with carbonyl carbons of gallic acid A (δ 164.19) and C (δ 164.19), respectively, indicating that two gallic acid units were connected as esters at C-6 positions of glucose A and B. The presence of the glycosidic linkage between C-1 of glucose A and C-3 of

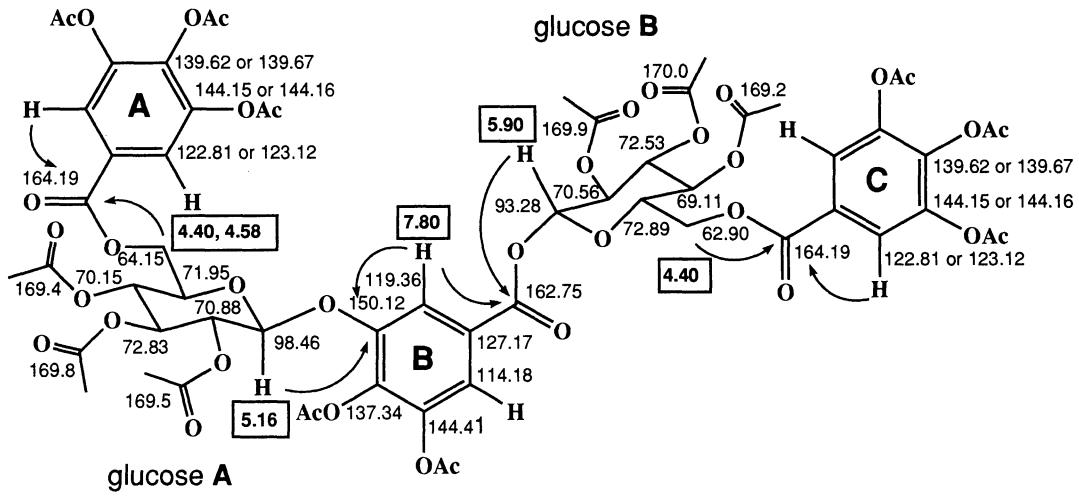

Fig. 2. ^{13}C NMR spectral data of **2** taken in C_6D_6; arrows show the correlation peaks in HMBC.

gallic acid B was evident by the observation of correlation peak between an anomeric proton of glucose A (δ 5.16) and C-3 carbon of gallic acid B (δ 150.12). Finally, the ester bond was obviously present at C-1 position of glucose B, by the observed correlation peaks between anomeric proton of glucose B (δ 5.90) and carbonyl carbon of gallic acid B (δ 162.75) , and H-2 of gallic acid (δ 7.80) and the carbonyl carbon of gallic acid B (δ 162.75). Furthermore, six carbonyl carbons of the acetates linked to two glucose units showed correlation peaks between the corresponding protons of glucose units.

Thus, the structure of new antioxidant, cyclamenoside (**1**) was determined and depicted as shown in Fig. 1. Antioxidant activity of cyclamenoside (**1**) was tested according to the method of Sodhi et al.[2] And inhibition of the release of hydrogen peroxide from peritoneal neutrophils of rat by cyclamenoside (**1**) was shown in Table 3. Activity of cyclamenoside (IC_{50} = 2.1 μM) was much stronger than those of the known compounds (quercetin and phenylbutazone; IC_{50} of both compounds = 15 μM). Cyclamenoside (**1**) also showed very weak but definite antibacterial activities against *Bacillus cereus* IFO 3001, *Micrococcus luteus* ATCC 9341, *Corynebacterium xerosis* 53-K-1, *Klebsiella pneumoniae* ATCC 10031, *Pseudomonas aeruginosa* IFO 3445, *Alcaligenes faecalis* IFO 13111, and *Flavobacterium meningosepticum* IFO 12535.

Table 3. Inhibition of hydrogen peroxide release from rat peritoneal neutrophils

Concentration / μM	40	20	10	5	2.5	1.25
Inhibition / %	91.0	95.5	96.7	91.2	68.4	13.9

The present study was partially supported by Yamada Science Foundation. The authors were grateful to Dr. Takao Nishi (Otsuka Pharmaceutical co., ltd.) for the measurement of antioxidant activities of cyclamenoside (**1**).

References

1) B. Havsteen, *Biochem. Pharmacol.*, **32**, 1141 (1983); R. A. Larson, *Phytochemistry*, **27**, 969 (1988).

2) A. Sodhi and P. Guputa, *Int. J. Immunopharmac.*, **8**, 709 (1986).

3) $[\alpha]_D^{20}$ -58.3(c 0.87 in methanol); positive FABMS: m/z 821(M+Na)$^+$; IR (film, cm^{-1}): 3389, 1697, 1612, 1344, 1226, 1070; ^{13}C NMR (CD_3OD): 168.41 (s), 168.35 (s), 166.81 (s), 146.84 (s), 146.81 (s), 146.39 (2 x s), 146.36 (2 x s), 142.44 (s), 139.80 (s), 139.76 (s), 121.33 (s), 121.30 (s), 120.77 (s), 113.86 (d), 112.27 (d), 110.45 (2 x d), 110.26 (2 x d), 104.16 (d), 96.27 (d), 77.92 (d), 77.29 (d), 76.32 (d), 75.91 (d), 74.76 (d), 74.03 (d), 71.31 (d), 71.25 (d), 64.57 (t), 64.45 (t).

4) Mp 144-145.5 °C; Elemental analysis: Found: C, 52.30; H, 4.56%. Calcd for $C_{61}H_{62}O_{37} \cdot H_2O$: C, 52.14; H, 4.59%; IR (film, cm^{-1}): 1778, 1755, 1601, 1496, 1429, 1373, 1327, 1201, 1057; ^1H NMR ($CDCl_3$) see Tables 1 and 2, signals for acetates: 1.98 (3H, s), 2.03 (6H, s), 2.04 (3H, s), 2.058 (3H, s), 2.061 (3H, s), 2.261 (6H, s), 2.263 (3H, s), 2.287 (9H, s), 2.29 (3H, s), 2.30 (3H, s); ^1H NMR (C_6D_6) see Tables 1 and 2, signals for acetates: 2.07 (3H, s), 1.95 (3H, s), 1.91 (3H, s), 1.83 (6H, s), 1.80 (3H, s), 1.764 (6H, s), 1.757 (3H, s), 1.723 (3H, s), 1.715 (3H, s), 1.688 (3H, s), 1.687 (3H, s), 1.53 (3H, s); ^{13}C NMR ($CDCl_3$) 170.13 (s), 170.07 (s), 169.70 (s), 169.64 (s), 169.29 (s), 167.79 (s), 167.53 (s x 2), 166.97 (s), 166.26 (s x 2), 163.98 (s), 163.94 (s), 162.37 (s), 149.45 (s), 143.68 (s), 143.01 (s x 2), 139.01 (s), 138.95 (s), 136.85 (s), 127.69 (s x 2), 126.71 (s), 122.59 (d x 2), 122.48 (d x 2), 119.31 (d), 113.87 (d), 98.36 (d), 92.73 (d), 72.56 (d), 72.46 (d), 72.14 (d), 71.68 (d), 70.52 (d), 69.96 (d), 68.83 (d), 68.54 (d), 63.18 (t), 62.88 (t), 20.84 (s), 20.63 (s), 20.59 (s), 20.56 (s), 20.51 (s x 3), 20.46 (s x 4), 20.10 (s x 2), 20.03 (s); ^{13}C NMR (C_6D_6) see Fig. 2, signals for methyl groups of acetates and carbonyl groups of aromatic acetates: 20.48 (q), 20.21 (q x 2), 20.12 (q), 20.08 (q), 20.01 (q x 2), 19.90 (q x 2), 19.88 (q x 2), 19.80 (q), 19.66 (q), 19.56 (q), 167.4 (s), 167.2 (s x 2), 167.1 (s x 2), 166.8 (s), 166.3 (s), 165.9 (s).

5) By the comparison of the spectral data (IR and ^1H NMR) with an authentic sample, both sugar parts were proved to be glucose.

(Received August 13, 1992)