Indonesian Medicinal Plants. XXIV.[1] Stereochemical Structure of Perseitol·K^+ Complex Isolated from the Leaves of *Scurrula fusca* (Loranthaceae)

Takashi ISHIZU, Hendig WINARNO, Etsuji TSUJINO, Tetsuo MORITA, and Hirotaka SHIBUYA*

Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Sanzo, 1 Gakuen-cho, Fukuyama, Hiroshima 729–0292, Japan. Received November 7, 2001; accepted January 8, 2002

A complex of perseitol (D-glycero-D-galacto-heptitol) and K^+ ions in a molar ratio of 20 : 1 was isolated from the leaves of *Scurrula fusca* (Loranthaceae), which has been traditionally used for the treatment of cancer in Sulawesi Island, Indonesia. The stereochemical structure of the complex in H_2O solution has been elucidated by use of several kinds of NMR techniques. Furthermore, it has been found that the complex exhibits a potent inhibitory effect on [^{3}H]-leucine incorporation for protein synthesis in Ehrlich ascites tumor cells in mice.

Key words *Scurrula fusca*; perseitol; potassium ion; complex; Indonesian medicinal plant; Loranthaceae

The parasite plant *Scurrula fusca* (BL.) G. DON. (Loranthaceae), whose host-plant is *Ficus riedelii* MIQ. (Moraceae), is locally called benalu alus, and the decoction of its leaves has been traditionally used to cure cancer in Sulawesi Island, Indonesia.

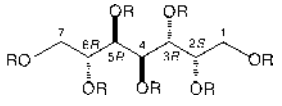
After cooling, from the methanol extract of the dried leaves were obtained amorphous precipitates, which were purified by HPLC on a gel partition resin to afford a complex (**1**) of perseitol and K^+ ions in 1.0% yield. This paper deals with the structural elucidation of the complex (**1**)[2] and its biological activity.

The complex (**1**), an amorphous powder, shows physicochemical properties of a melting point at 174—177 °C and specific rotation, $[\alpha]_D$ −2.0° (H_2O). Acetylation of the complex (**1**) with pyridine and acetic anhydride furnished an acetate (**2**), which was identified as perseitol heptaacetate[3] by comparison of physicochemical properties including its specific rotation $[\alpha]_D$ −14.8° (MeOH) with those in the literature.[3] Therefore, it has been found that the organic part of **1** is perseitol (D-glycero-D-galacto-heptitol).

Fluorescence X-ray analysis indicated that the complex (**1**) contained K^+ ions and did not have other metal ions. Atomic absorption analysis showed that the complex (**1**) consisted of perseitol (**1a**) and K^+ ions in a molar ratio of 20 : 1.

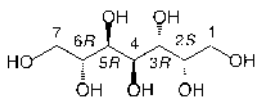
In order to clarify the stereochemical structure of the complex (**1**) in H_2O solution, detailed NMR analysis of **1a** and **1** was performed. The conformation of perseitol (**1a**) in H_2O solution was found to be a "zigzag" with a planar carbon chain by using a Kurplus type equation concerning heptitol between the coupling constants and dihedral angles.[4—6] Furthermore, no significant change in chemical shift or signal breadth in the ^{1}H- and ^{13}C-NMR spectra of **1a** was observed at 1.67×10^{-2}—1.67×10^{0} M in H_2O, which indicates that self-assembly did not occur between those concentration ranges.

Next, we investigated changes in the ^{1}H-NMR signal breadth of perseitol (**1a**) at 1.67×10^{-1} M solution in H_2O by adding regular amounts of KSCN as a potassium source to adjust the molar ratio of **1a** to the K^+ ion from 26 : 1 to 10 : 1 (Fig. 2). The proton signals of **1a** gradually broadened with increases in the amount of K^+ ions, from 26 : 1 to 20 : 1, and the signal breadth was widest at a molar ratio of 20 : 1. These findings indicated that the proton motions of perseitol (**1a**) were restricted due to complex formation with the K^+ ions, and that 20 molecules of **1a** formed a complex (**1**) with one



R=H Peresitol (D-glycero-D-galacto-heptitol) (**1a**)
R=Ac Peresitol heptaacetate (**2**)

Fig. 1



Perseitol

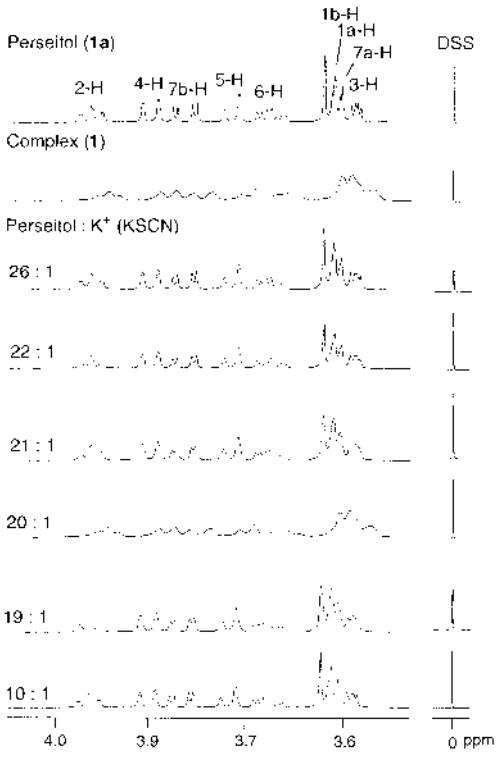


Fig. 2. Changes in ^{1}H-NMR Signals of Perseitol (**1a**) (10.61 mg, 1.67×10^{-1} M) by Adding KSCN

* To whom correspondence should be addressed. e-mail: shibuya@fupharm.fukuyama-u.ac.jp

mole of the K^+ ion in H_2O solution state. Thereafter, the widening proton signals of **1a** became sharper again, from 20 : 1 to 10 : 1, due to the untying of the complex (**1**).

The ^{1}H-NMR spectrum of the complex (**1**) gave a similar signal pattern to that of perseitol (**1a**), except for the signal breadth, which indicates that the planar "zigzag" conformation of **1a** is also maintained in the complex (**1**).

Figure 3 shows plots for the chemical shifts of methine and methylene proton signals of perseitol (**1a**) against a molar ratio of **1a** and K^+ ions. The result indicated that every proton signal at a molar ratio of 20 : 1 shifted upfield most prominently. Therefore, the stoichiometry in complex (**1**) of perseitol (**1a**) with the K^+ ion was confirmed to be 20 : 1.

Furthermore, changes in chemical shifts of the seven hydroxyl proton signals of 1.67×10^{-1} M solution of perseitol (**1a**) in DMSO-d_6 were observed by adding regular amounts of KSCN to adjust the molar ratio of K^+ ion to **1a** from 0 : 1 to 1 : 1 (Fig. 4). The 3-hydroxyl proton signal shifted downfield most prominently, before the 2-, 4-, and 5-hydroxyl proton signals shifted. The downfield shifts of 1-, 6-, and 7-hydroxyl proton signals were not as prominent as those of other hydroxyl proton signals. These facts indicated that the 3-hydroxyl group of perseitol (**1a**) most strongly attracted the K^+ ions in forming complex (**1**), while the 2-, 4-, and 5-hydroxyl groups attracted these ions more strongly than the 1-, 6-, and 7-hydroxyl groups. The tendency of a downfield shift of the seven hydroxyl protons was similarly observed as in the case of a molar ratio of 20 : 1 (KSCN/perseitol = 0.05) (Fig. 5).

As a result, it may be presumed that 20 molecules of perseitol (**1a**), while maintaining the "zigzag" conformation, associate with the surrounding K^+ ions by the above-mentioned interaction (Fig. 5) to form the complex (**1**), which has a spherical stereochemical structure in H_2O solution. Furthermore, the supposition that the spherical stereochemical structure was composed of one layer of perseitol (**1a**) (Fig. 3) was accounted for by the one-signal pattern in the ^{1}H-NMR spectrum of the complex (**1**).

The aqueous solution containing persitol (**1a**) and K^+ ions in a molar ratio of 20 : 1, in fact, gave complex (**1**) as an amorphous powder, with physicochemical properties including melting point and specific rotation which were identical with those of complex (**1**) isolated from the dried leaves of *Scurrula fusca*. However, the molecular weight of complex (**1**) was not detected by several kinds of MS techniques, *i.e.* EI, FAB, ESI, and TOF.

We also investigated the interaction between perseitol (**1a**) and other metal ions, Na^+ from NaSCN, Mg^{2+} from $MgCO_3$, Ca^{2+} from $Ca(SCN)_2$, and Ag^+ from Ag_2CO_3, by the same ^{1}H-NMR analysis. However, no combination afforded a similar complex in H_2O solution. It was therefore considered that a cation size of K^+ ion might be the essential factor in the formation of complex (**1**).

It should be noted that complex (**1**) exhibits an inhibitory effect on protein synthesis in Ehrlich ascites tumor cells in

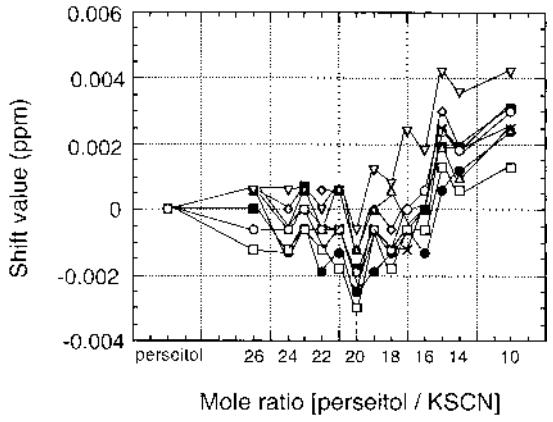


Fig. 3. Shifts in ^{1}H-NMR Signals of Methine and Methylene Protons of Perseitol (**1a**) (10.61 mg, 1.67×10^{-1} M) by Adding KSCN

1_{ab}-H (◇), 2-H (■), 3-H (○), 4-H (●), 5-H (▽), 6-H (×), 7a-H (□), 7b-H (△).

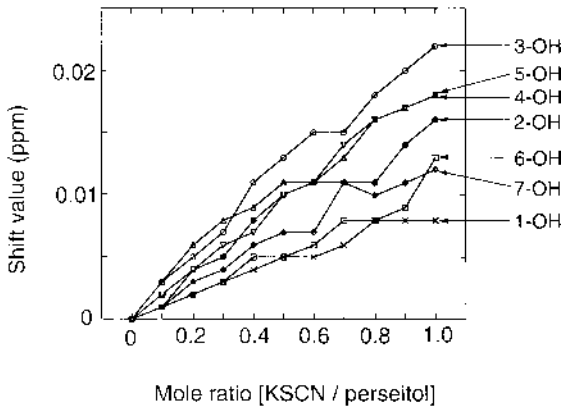


Fig. 4. Shifts in ^{1}H-NMR Signals of Hydroxyl Protons of Perseitol (**1a**) (10.61 mg, 1.67×10^{-1} M) by Adding KSCN

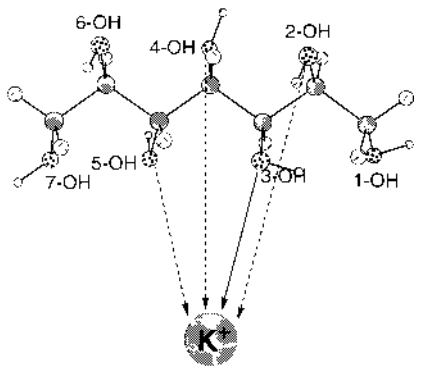


Fig. 5

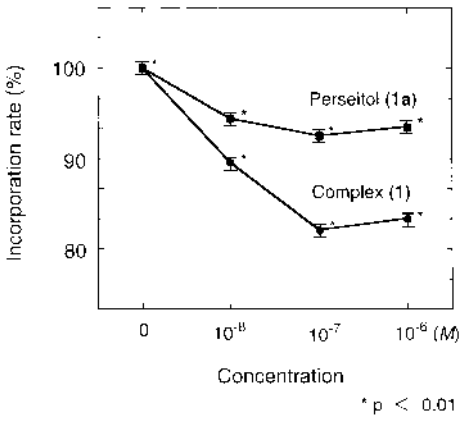


Fig. 6. Changes in Incorporation Rates of [^{3}H]-Leucine into Ehrlich Ascites Tumor Cells by Complex (**1**) and Perseitol (**1a**)

The molecular weight of complex (**1**) was calculated to be 212 (**1a**).

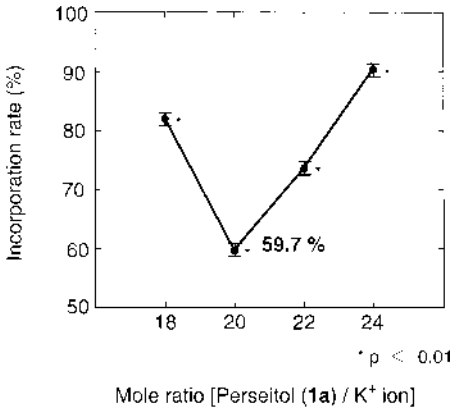


Fig. 7. Changes in Incorporation Rates of [^{3}H]-Leucine into Ehrlich Ascites Tumor Cells by the Various Mole Ratios of Perseitol (**1a**) and K^+ Ions (at 10^{-4} M)

mice. Figure 6 shows plots for [^{3}H]-leucine incorporation rates into the Ehrlich ascites tumor cells[7] by administrating complex (**1**) and K^+ ion free perseitol (**1a**).

Furthermore, [^{3}H]-leucine incorporation rates into the Ehrlich ascites tumor cells in the presence of aqueous solutions containing the various mole ratios of perseitol (**1a**) and K^+ ions were also investigated (Fig. 7). The solution of the molar ratio 20 : 1 most strongly inhibited the protein synthesis, with 40.3% inhibition at 10^{-4} M. However, complex (**1**) and perseitol (**1a**) did not show cytotoxicity against L1210 cells.

It was therefore concluded that complex (**1**) of perseitol and K^+ ions in a molar ratio 20 : 1 might be one of the biologically active substances in the leaves of *Scurrula fusca* (Loranthaceae).

Experimental

^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JMN-LA 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer. ^{1}H and ^{13}C chemical shifts (δ) are quoted in ppm relative to sodium 2, 2-dimethyl-2-silapentane-5-sulfate (DSS, δ=0) in H_2O and tetramethylsilane (TMS, δ=0) in DMSO-d_6 as an internal standard, and coupling constants are given in Hz. The measuring temperature was 25.0 °C. The following abbreviations were used: singlet (s), doublet (d), double doublet (dd), double double doublet (ddd), triplet (t), quartet (q), multiplet (m), and broad signal (br).

HPLC was carried out with a Shimadzu LC-10AD. Melting points were measured on a Yanagimoto micro hot-stage melting point apparatus without correction. Optical rotations were measured with a JASCO DIP-360 automatic polarimeter. FAB-MS was obtained with a JEOL JMS-DX 300L spectrometer using glycerol as a matrix. Fluorescence X-ray was obtained with a Rigaku-Kevex energy-dispersive X-ray spectrometer (ultra-trace system). Atomic absorption was recorded on a Shimadzu atomic absorption flame emission spectrophotometer AA-670.

Plant Material *Scurrula fusca* (Loranthaceae) was collected in the Poso area of Sulawesi Island, Indonesia, in August, 1992, and was identified in the Herbarium Bogoriense, Research Centre for Biology-LIPI, Indonesia.

Sample Tube for NMR Measurements A double sample tube system for measurements in H_2O was used. Inside: an NMR sample tube (ϕ 3 mm) contained D_2O (200 μl) for a field-frequency lock, and DSS (0.1 mg) was used as the internal reference. Outside: an NMR sample tube (ϕ 5 mm) contained H_2O (300 μl) as the solvent, plus sample (0.05 mmol).

Isolation of the Complex (1) The dried leaves (250 g) of *Scurrula fusca* (Loranthaceae) were extracted three times with methanol (each 500 ml) under reflux for 3 h. The combined extract solution was filtered, and the filtrate was cooled to room temperature to give precipitates (12.5 g). The precipitates were collected and stirred in purified water for 24 h. The resulting insolubility was removed by filtration and the filtrate was lyophilized to give an amorphous powder, which was purified by HPLC [column, TSK-GEL G-3000 PW XL (TOSOH); elution, purified water] to afford the complex (**1**) of perseitol and K^+ ions (20 : 1) (**1**, 2.5 g, 1.0% from the dried leaves). The complex of perseitol and K^+ ions (20 : 1) (**1**): an amorphous powder, mp 174—177 °C, $[\alpha]_D$ −2.0° (c=3.0, in H_2O at 25 °C). IR (KBr) cm^{-1}: 3284. ^{1}H-NMR (H_2O) all protons of **1** appeared as broadening signal. δ: 3.64 (1H, 3-H), 3.65 (1H, 7-Ha), 3.68 (2H, 1-Ha, 1-Hb), 3.75 (1H, 6-H), 3.79 (1H, 5-H), 3.85 (1H, 7-Hb), 3.89 (1H, 4-H), 3.97 (1H, 2-H). ^{13}C-NMR (D_2O) δ_C: 66.2 (1-C), 66.2 (7-C), 71.2 (4-C), 72.1 (5-C), 72.2 (3-C), 73.2 (2-C), 73.9 (6-C). ^{1}H-NMR (D_2O) all protons of **1** appeared as broadening signal. δ: 3.66 (1H, 3-H), 3.67 (1H, 7-Ha), 3.68—3.69 (2H, 1-Ha, 1-Hb), 3.77 (1H, 6-H), 3.81 (1H, 5-H), 3.87 (1H, 7-Hb), 3.91 (1H, 4-H), 3.98 (1H, 2-H). ^{13}C-NMR (D_2O) δ_C: 66.0 (1-C), 66.0 (7-C), 71.0 (4-C), 71.9 (5-C), 72.0 (3-C), 73.0 (2-C), 73.7 (6-C). FAB-MS *m/z*: 213 [highest peak, (perseitol+H)$^+$]. *Anal.* Calcd for $C_{140}H_{320}O_{140}K\cdot 5H_2O$: C, 38.45; H, 7.61. Found: C, 38.23; H, 7.53.

NMR Spectrum of Perseitol (**1a**): ^{1}H-NMR (H_2O) δ: 3.64 (1H, dd, $J_{3,4}$=9.5 Hz, 3-H), 3.65 (1H, dd, $J_{6,7a}$=5.8 Hz, $J_{7a,7b}$=11.9 Hz, 7-Ha), 3.68 (2H, m, 1-Ha, 1-Hb), 3.75 (1H, ddd, $J_{6,7b}$=2.7 Hz, 6-H), 3.79 (1H, dd, $J_{5,6}$=8.8 Hz, 5-H), 3.85 (1H, dd, 7-Hb), 3.90 (1H, dd, $J_{4,5}$=1.0 Hz, 4-H), 3.97 (1H, dd, $J_{2,3}$=1.2 Hz, 2-H). ^{1}H-NMR (DMSO-d_6) δ: 3.37 (1H, m, 7-Ha), 3.40 (2H, m, 1-Ha, 1-Hb), 3.45 (1H, m, 3-H), 3.45 (1H, m, 6-H), 3.57 (1H, m, 5-H), 3.61 (1H, m, 7-Hb), 3.68 (1H, m, 4-H), 3.71 (1H, m, 2-H), 3.95 (1H, d, J=7.6 Hz, 3-OH), 4.02 (1H, d, J=7.9 Hz, 5-OH), 4.06 (1H, d, J=7.3 Hz, 4-OH), 4.12 (1H, d, J=5.5 Hz, 2-OH); 4.34 (1H, dd, J=5.5, 5.8 Hz, 7-OH), 4.40 (1H, d, J=6.7 Hz, 6-OH), 4.43 (1H, dd, J=5.5, 5.8 Hz, 1-OH). ^{13}C-NMR (DMSO-d_6) δ_C: 63.1 (7-C), 63.8 (1-C), 68.4 (5-C), 69.0 (4-C), 69.5 (5-C), 70.0 (6-C), 71.5 (2-C). The ^{1}H- and ^{13}C-NMR spectra in D_2O were identical with those in the literature.[6]

Preparation of the Complex (1) from Perseitol (1a) and K^+ Ions (20 : 1) A solution of perseitol (**1a**, 10.6 mg, 0.05 mmol) and KSCN (0.24 mg, 0.0025 mmol) in H_2O (300 μl), when widening proton signals were observed in the ^{1}H-NMR spectrum, was lyophilized to give an amorphous powder. The powder was purified by HPLC [column, TSK-GEL G-3000 PW XL (TOSOH); elution, purified water] to afford the complex (**1**) of perseitol and K^+ ions (20 : 1) (**1**, 2.2 mg, 1.0%), which was identified with the complex isolated from the leaves of *Scurrula fusca* by comparison of physicochemical data, including the specific rotation.

The Interaction between Perseitol (1a) and Na^+, Mg^{2+} Ca^{2+}, and Ag^+ Ions To the solution of perseitol (**1a**, 10.6 mg, 0.05 mmol) in H_2O (300 μl) was added regular amounts of NaSCN, $MgCO_3$, $Ca(SCN)_2$, and Ag_2CO_3, adjusting the molar ratio of **1a** to the Na^+, Mg^{2+}, Ca^{2+} and Ag^+ ions from 26 : 1 to 10 : 1, respectively. In every case, the breadth of the ^{1}H-NMR signals assignable to **1a** was not changed, indicating no formation of a complex of perseitol (**1a**) and the metal cations in H_2O solution.

Acetylation of the Complex (1) A solution of complex (**1**, 143 mg) in pyridine (0.5 ml) was treated with Ac_2O (1.0 ml) and left at room temperature for 2 h. The solvent was removed *in vacuo* to give a residue, which was purified by column chromatography on silica gel and eluted with *n*-hexane-ethyl acetate (3 : 2) to give perseitol heptaacetate (**2**, 150 mg, 44%). Perseitol heptaacetate (**2**): colorless needles from diethyl ether, mp 119—120 °C, $[\alpha]_D$ −14.8° (c=1.2, in MeOH at 25 °C). IR (KBr) cm^{-1}: 1749. ^{13}C-NMR ($CDCl_3$) δ_C: 20.5, 20.6, 20.7, 20.8 (totally 7C, –$OCOCH_3$×7), 61.8, 62.1 (1-C, 7-C), 66.5, 67.1, 67.5, 67.6, 67.9 (4-, 3-, 5-, 2-, 6-C), 169.5, 169.8, 170.1, 170.3, 170.4 (totally 7C, –$OCOCH_3$×7). FAB-MS *m/z*: 507 (M+H)$^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{21}H_{31}O_{14}$: 507.1713. Found: 507.1711 (M+H)$^+$. The ^{1}H-NMR spectrum was identical with that in the literature.[3]

Determination of [^{3}H]-Leucine Incorporation Rate Samples at the indicated concentrations were dissolved into the suspension of [^{3}H]-leucine (18.5 kBq/dish) in Ehrlich ascites tumor cells in mice which had been incubated in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 5.0 mM glucose and 2.0% albumin for 30 min. The incubated Ehrlich ascites tumor cells were washed twice with ice-cold 50 mM Tris–HCl buffer (pH 7.4) and homogenized in 0.5 ml of the same buffer for 10 s by sonication. The homogenate was spotted on a strip of filter paper (1.5×1.5 cm, Whatmann, 3MM) and treated by the TCA-precipitate method to obtain the radioactivity.[8,9]

Acknowledgements This work was partially supported by Grants-in-Aid for International Scientific Research (No. 02041054 and No. 04041069) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1) Part XXIII: Ohashi K., Bohgaki T., Matsubara T., Shibuya H., *Chem. Pharm. Bull.*, **48**, 433—435 (2000).

2) Preliminary communication: Ishizu T., Tsujino E., Winarno H., Ohashi K., Shibuya H., *Tetrahedron Lett*., **42**, 6887—6889 (2001).
3) Moore R. E., Barchi J. J., Jr., Bartolini G., *J. Org. Chem*., **50**, 374—379 (1985).
4) Karplus M., *J. Chem. Phys*., **30**, 11—15 (1959).
5) Haasnoot C. A. G., Leeuw F. A. A. M. DE., Altona C., *Tetrahedron*, **36**, 2783—2792 (1980).
6) Lewis D., *J. Chem. Soc. Perkin Trans II*, **1986**, 467—470.
7) Morita T., Sakata K., Kanagawa A., Ueki H., *Biol. Pharm. Bull*., **17**, 577—580 (1994).
8) Ashyby P., Bennett D. P., Spencer I. M., Robinson D. S., *Biochem. J*., **176**, 865—872 (1978).
9) Sera M., Tanaka K., Morita T., Ueki H., *Arch. Biochem. Biophys*., **279**, 291—297 (1990).