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9. Tyunosin Ukita*1 and Akio Tanimura*2 : Studies on the Anti-tumor Component in the Seeds of *Coix Lachryma-Jobi* L. VAR. *Ma-yuen* (ROMAN.) STAPF. I. Isolation and Anti-tumor Activity of Coixenolide.

(*Faculty of Pharmaceutical Sciences, University of Tokyo*[*1])

Since the ancient time, Chinese pearl barley (薏苡仁) (seeds of *Coix Lachryma-Jobi* L. VAR. *Ma-yuen* (ROMAN.) STAPF) has been used as a remedy in the East, and its seeds and roots have been applied as a diuretic, stomachic, nourishment, anodyne, and antispasmodic. However, only a few detailed studies are found on their effective constituents.[1~3]

Steroids,[4] fats[5~7] and constituent amino acids,[8] and especially *cis*-8-octadecenoic acid[9] accompanied with saturated fatty acids such as palmitic acid and stearic acid, have been found in the seeds. On the other hand, coixol was isolated from its roots and found to have anodynic and antispasmodic activities.[10]

Ukita and Tsumita[11] in their investigation on the anti-cancer activity of natural and synthetic compounds, found that the acetone extract of pearl barley shows a growth inhibiting activity on Ehrlich ascites sarcoma of mouse.

Further, Ukita and Iguchi[12] observed that the main activity of the acetone extract included an acid substance soluble in petroleum ether. This activity of acetone extract of the seeds was confirmed by Umezawa and Takeuchi.[13]

The present paper deals with a more detailed investigation on the anti-tumor component in the acetone extract, resulting in isolation of a new component, coixenolide, which showed the highest anti-tumor activity to Ehrlich ascites sarcoma in mice, among other constituents of the seeds.

TABLE I. Biological Test of Extracts for Growth Inhibiting Action on Ehrlich Ascites Tumor Cells in Mice

Organic solvent used for extraction	Dose of the extract given (mg./day/mouse)	Result after 7 days of application (No. died/Total no. of mice used)	
		original	×2 dilution
Petroleum ether	8.8	2/2	2/2
Ether	9.7	2/2	2/2
Acetone	10.3	0/2	0/2
Chloroform	8.2	0/2	2/2
Methanol	6.1	1/2	2/2
Ethyl acetate	8.4	0/2	2/2

*1 Hongo, Tokyo (浮田忠之進).
*2 Present address : National Institute of Hygienic Sciences, Tamagawa-Yoga-machi, Setagaya-ku, Tokyo (谷村顕雄).

1) K. Yoshimura : Nippon Kagaku Zasshi **40**, 483 (1919).
2) G. Vallaets : Bull. Agr. Congo Belge, **39**, 247 (1948).
3) M. H. French : E. Afr. Agr. J. Kenya, **10**, 152 (1945).
4) S. Kuwada : Yakugaku Zasshi, **59**, 560 (1939).
5) F. Berger : Scientica Pharm., **11**, 122 (1938).
6) R. H. Willey, P. H. Wilken : J. Org. Chem., **16**, 1536 (1951).
7) K. Hano, K. Ohtsu : Yakugaku Zasshi, **79**, 1412 (1959).
8) G. Hattori : J. Biochem. (Tokyo), **1**, 365 (1922).
9) Y. Matsushima : Folia Pharmacol. Japon., **48**, 109 (1952).
10) T. Koyama : Yakugaku Zasshi, **75**, 699, 702 (1955).
11) T. Ukita, T. Tsumita, *et al.* : Gann, **40**, 140 (1949).
12) T. Ukita, S. Iguchi, *et al.* : *Ibid.*, **41**, 91 (1950).
13) H. Umezawa, T. Takeuchi : Private communication.

Pearl barley was successively extracted with six kinds of organic solvents; petroleum ether, ether, acetone, ethyl acetate, chloroform, and methanol, and each extract was examined for growth inhibiting action against Ehrlich ascites tumor cells in mice (Table I).

From the result of biological tests, acetone extract was found to be the most effective, and ethyl acetate and chloroform extracts were slightly effective, while other extracts were found ineffective. For further purification of the active component, the acetone extract was put on top of a silica gel column and eluted successively with five kinds of organic solvents, petroleum ether, ether, acetone, ethyl acetate, and methanol (Chart 1).

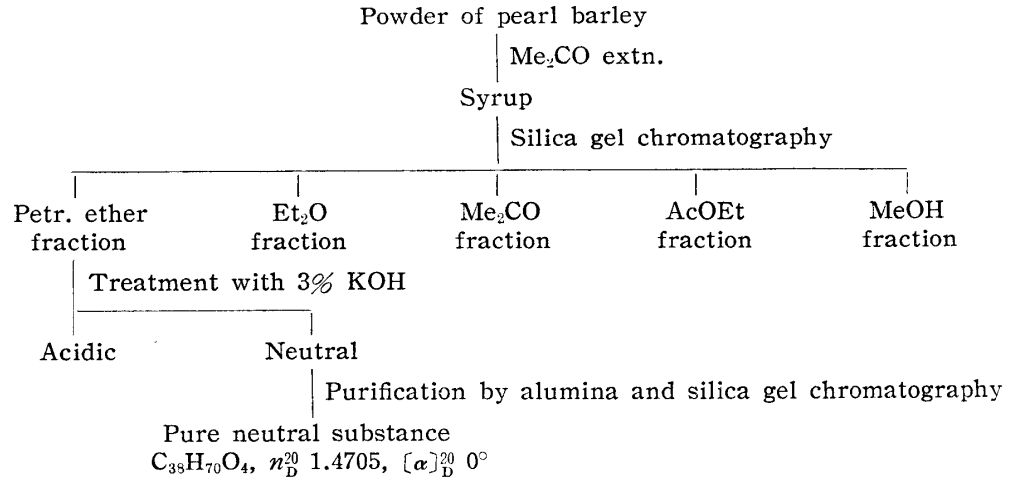

Chart 1. Isolation of Pure Neutral Substance from Pearl Barley

Each fraction was examined by biological tests and only the petroleum ether eluate was found to be effective. When the solvent was evaporated, the petroleum ether fraction left a yellow oil. Neutral component of this fraction was separated from acidic substances and the former was found to have the most of the growth inhibiting activity on the ascites sarcoma, while the latter showed only a slight effect (Fig. 1).

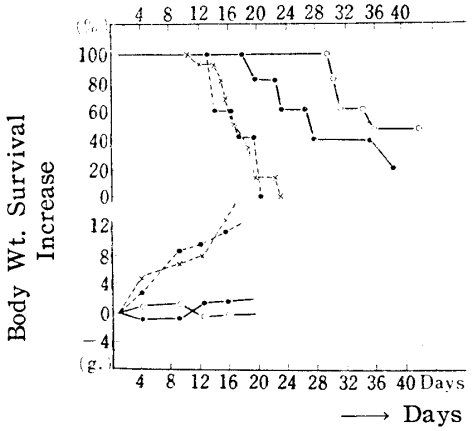

Fig. 1. Anti-tumor Activities of Extracts from Pearl Barley against Ehrlich Ascites Sarcoma in Mouse

—○—○— Acetone extract
—•—•— Neutral oil
---•---•--- Mixture of acid substances
---×---×--- Control

The neutral fraction was further purified by a column chromatography through alumina treated with Bromothymol Blue, for the purpose of separating the contaminating acid substances.

The absolutely neutral component thus obtained did not boil out even at 220° under 10^{-4} mm. Hg, but colored slightly. Further purification of this neutral fraction was made

by a column chromatography reported by Fillerup,[14] using silica gel as the stationary phase and petroleum ether–ether mixtures (4%, 10%, and 50% ether in petroleum ether) as the elution solvent. By this procedure this fraction was separated into two compounds, A and B, the result of which is given in Fig. 2.

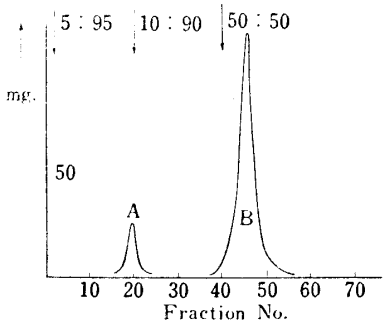

Fig. 2. Purification of the Neutral Substance by Fillerup's Column Chromatography

Adsorbent : Silica gel
Solvent : Ether-petr. ether mixture

Among these compounds, only B was found to be biologically active. The compound B gave a single peak in further repeated fractionation by Fillerup's column chromatography, using varied solvent systems, and was analysed to give a molecular formula of $C_{38}H_{70}O_4$, and physical constants of n_D^{20} 1.4705, $[\alpha]_D^{20}$ 0°. As will be reported in the following paper of this series, examination of this purified compound, named coixenolide, revealed that it had the structure of 1-methyl-2-(*cis*-9-hexadecenoyloxy)propyl *trans*-11-octadecenoate.

Experimental

Acetone Extraction of Pearl Barley—Dried and powdered Chinese pearl barley (2 kg.) was extracted three times with Me_2CO (5 L.) at room temperature. The extracts were combined and concentrated *in vacuo* to give 86.6 g. of a red-brown syrup. This syrup was dissolved in 450 cc. of petr. ether, filtered, and the filtrate was again concentrated. The yield of red-brown syrup was 85.0 g.

Separation of a Fraction Active in Growth Inhibition of Ehrlich Ascites Sarcoma in Mouse—A solution of 20 g. of Me_2CO extract dissolved in 60 cc. of petr. ether was placed on the top of a silica gel column (60×450 mm.) and eluted first with 1000 cc. of petr. ether, and then successively with 500 cc. each of Et_2O, Me_2CO, AcOEt, and MeOH. The fractions obtained for each solvent were combined and the solvent was evaporated to give petr. ether fraction (3.75 g.), Et_2O fraction (13.0 g.), Me_2CO fraction (1.5 g.), AcOEt fraction (0.6 g.) and MeOH fraction (0.1 g.). Each fraction was used for inhibition test against Ehrlich sarcoma in mice. Only petr. ether fraction was active.

Purification of Petroleum Ether Fraction—A solution of 15 g. of the petr. ether fraction dissolved in 60 cc. of petr. ether was shaken twice with 0.2*N* KOH solution to separate into acid (11.7 g.) and alkali-insoluble neutral portion (3.0 g.). According to the result of biological test, the neutral part was active and acidic substances showed only a slightly inhibiting action against the ascites sarcoma. For a complete separation of contaminating acidic substances in the neutral portion, column chromatography through alumina treated with Bromothymol Blue was employed. The column was prepared with alumina presteeped in 1% $CHCl_3$ solution of Bromothymol Blue. A solution of 3 g. of the neutral substances dissolved in 10 cc. of $CHCl_3$ was passed through the column of alumina and eluted with $CHCl_3$. The elution was continued until the yellowed band of Bromothymol Blue-alumina reached bottom of the column. After all of the alumina colored yellow, the receiver flask was changed and elution was continued with Me_2CO till yellow color returned to blue. The $CHCl_3$ eluate contained the neutral substance and Me_2CO eluate contained the acidic substances, and the neutral substances were separated completely from the acidic substances. On removal of the solvent, $CHCl_3$ fraction gave 2.4 g. of a pale yellow oil and Me_2CO fraction gave 0.52 g. of a yellow oil.

Further Purification of Neutral Substances—a) Vacuum distillation : In order to purify the neutral oil isolated from the seeds, vacuum distillation was applied, but no distillate was obtained at 220° under 10^{-4} mm. Hg.

b) Chromatography : According to the Fillerup's method, silica gel column chromatography was employed. Silica gel was packed in a column (10×350 mm.) with petr. ether and a solution of 250 mg. of the neutral substance dissolved in 6 cc. of petr. ether was passed through the column. For elution,

14) D. L. Fillerup, J. F. Mead : Proc. Soc. Exptl. Biol. Med., **83**, 574 (1953).

three kinds of mixed solvents of Et_2O-petr. ether of 4:96 v/v% (100 cc.), 10:90 v/v% (100 cc.), and 50:50 v/v% (200 cc.) were successively used. Effluents were collected in 5-cc. (60 drops) fractions. The solvent was removed and the residue was weighed to obtain distribution of the fractionated substances (Fig. 3). Fractions A and B were tested for anti-tumor activity against Ehrlich ascites sarcoma in mice and it was found that fraction A was inactive, while fraction B was active in this biological test. *Anal.* Calcd. for $C_{38}H_{70}O_4$: C, 77.23; H, 11.94. Found (Fraction B) : C, 77.02; H, 11.80. n_D^{20} : 1.4705, $[\alpha]_D^{20}$: 0°. This compound was named coixenolide.

Biological Test—In the *in vivo* test against Ehrlich ascites sarcoma in mice, 2×10^6 of the ascites sarcoma cells were injected intraperitoneally in a control and experimental groups of mice. The fractions or compounds to be tested were given to the experimental group intraperitoneally 2 hr. after transplantation for following 7 days. Effect of the test material was estimated by the difference in body weight between the control and experimental group, and the difference in the survival time between the two groups.

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Summary

From the acetone extract of pearl barley, seeds of *Coix Lachryma-jobi* L. VAR. *Mayuen* (ROMAN.) STAPF, a new compound, named coixenolide, was isolated. This compound, $C_{38}H_{70}O_4$, showed a growth-inhibiting action against Ehrlich ascites sarcoma cells in mice.

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