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Address: Prof. Dr. Krishna C. Joshi, Department of Chemistry, University of Rajasthan, Jaipur-302004, India

Faculty of Pharmaceutical Sciences, Kyushu University, National Cancer Center Research Institute, Japan***

ALOE MANNAN, POLYSACCHARIDE, FROM ALOE *ARBORESCENS* VAR. *NATALENSIS*

By A. YAGI, K. MAKINO, I. NISHIOKA* and Y. KUCHINO**

Abstract

A main polysaccharide (aloe mannan) isolated from the fresh leaf pulp (aquiferous tissue) of Aloe arborescens MILL. *var. natalensis* BERGER *in a pure state was proved to be a partially acetylated β-D-mannan. The molecular weight of aloe mannan was calculated to be approximately 15,000 by equilibrium ultracentrifugation. An inhibiting effect of aloe mannan was tested against the implanted sarcoma-180.*

Introduction

The fresh leaves of *Aloe spp.* were widely used as folk medicines [1], and have been applied for disease such as peptic ulcers [2], leg ulcers [3], X-ray burns [4] and for ornamentals [5], while much of the commercial Aloe or aloin were valued as laxatives. On the studies of the pulp of *Aloe vera* L., ROBOZ [6] reported the isolation of a polysaccharide consisting of about equal part of D-glucose and D-mannose together with a small amount of uronic acid. Recently, OVODOVA [7] isolated a polysaccharide composed of galacturonic acid from *Aloe arborescens* MILL.

Among several species of *Aloe* in Japan, *Aloe arborescens* MILL. *var. natalensis* BERGER was widely cultivated in the South and has been applied as a folk remedy for various external and internal disease.

In view of the medicinal significance the studies on the phenolics in the green assimilatory of this plant were carried out to give aloenin (aloearbonaside), barbaloin [8-a], 2"-O-feruloylaloesin, 2"-O-*p*-coumaroylaloesin [8-b]. On the studies of the fresh leaf pulp we isolated a polysaccharide, named aloe mannan, as a pure state. This paper deals with the characterization of aloe mannan indicating activity for the implanted sarcoma-180 in mouse.

Results and Discussion

The geratinous pulp was removed from the green leaves mechanically, and the

ot water-extract of the pulp was purified by dialysis, deproteinization and epeated precipitation from acetone-water to afford a fibrous white compound A.

Compound A showed that it contained neither phenolic components nor starch y $FeCl_3$, benzidine and iodine tests. On infra red (IR) spectrum compound A ndicated carbonyl absorption band at 1735 cm^{-1}, and revealed a characteristic cetyl methyl signal at δ 2.18 on nuclear magnetic resonance (NMR) spectrum [9].

Since compound A in 0.1 N NaCl solution presented a single symmetrical peak n the sedimentation diagram ($S_{20,w}$=1.55 S, Fig. 1) it was proved to be homo-eneous. The average molecular weight was estimated to be approximately 15,000 y the sedimentation equilibrium method (Archibald method) [10], when the artial specific volume, was assumed to be that of D-glucose, 0.621 [11].

In the assay of anti-tumor activity, compound A showed effectiveness for the mplanted sarcoma-180 in mouse, and no toxicity was observed (Table I).

Fig. 1. Sedimentation Patterns of Compound A in 0.1 N NaCl solution. Exposures were made every 30 minutes, after 52,640 rpm had reached. In these cases sedimentation is from right to left. Temperature: 20°

Table I
Anti-tumor Effect[a] of Compound A

Sample	Dose mg/kg xday	Inhibition ratio (%)	Complete regression	Mortality (died/total)	Average body wt. change (g)	Av. wt. of tumor (g)
Comp. A						
	5×10	38.1	2/10	1/10	+ 4.68	7.75
	100×10	48.1	1/10	0/10	+ 7.60	6.50
Control	-		1/10	1/10	+ 3.57	12.53

a) tumor, sarcoma-180; animal, mouse (ICR); route, i.p. vehicle, aq.dest.

By saponification compound A yielded an acid and compound B, which showed no carbonyl absorption band on IR spectrum. The acid liberated was identified to acetic acid by direct comparison through the *p*-phenylphenacyl acetate. The IR spectrum of compound B showed the similar absorption bands to those of a known plant β-1,4-linked D-mannan [12]. On hydrolysis using 5% H_2SO_4 compound B gave only D-mannose. Thus, compound B was determined to be β-D-mannan.

From above evidence compound A, named aloe mannan, was demonstrated to be a partially acetylated β-D-mannan.

It may be noteworthy that the water-soluble partially acetylated β-D-mannan presented the activity for the implanted salcoma-180 in mouse as well as yeast mannan [13], glucan [14] and acetylated glucan [9].

Experimental

Paper partition chromatography was carried out on Toyo-Roshi No. 51 with following solvent systems: a) BuOH-pyridine-H_2O (6:4:3), b) BuOH-AcOH-H_2O (4:1:5, upper layer). Spraying reagent used for detection was aniline hydrogen phthalate. The IR spectra were measured with KOKEN DS-301 and NMR spectrum was taken with Nihondenshi C-100 H in D_2O with sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DDS) as an internal standard at 100 ± 5°. A spinco Model E analytical ultracentrifuge with a schlieren optical system was used for determination of molecular weight. ORD spectrum was measured on a JASCO ORD/UV 5 spectrometer and optical rotation on JASCO DIP-SL automatic polarimeter.

Preparation of material: The fresh plant leaves (50 kg) were harvested from the Herbal garden of this University, and each of them was cut in half parallely. The colorless geratinous pulp was separated carefully by scraping from the green assimilatory tissue containing phenolic substances. The homogenized pulp (21 kg) was extracted with hot water and after filtration the water layer was concentrated to 500 ml under reduced pressure. The filtrate was adjusted with 1N HCl to pH 4.5–5.0 and dialyzed for one week against running water. To the non dialyzable layer $CHCl_3$ was added to removed protein by vigorous shaking. The suspension was centrifuged off and the supernatant was concentrated to 300 ml. To the crude viscous layer three volumes of acetone were added under ice cooling to exclude pale colored substances. The colored substance was filtrated off, and to the filtrate two volumes of acetone were added to precipitate the slightly grayish fibrous mass under ice cooling. The fibrous flakes dissolved in hot water were purified by repeated precipitation with acetone to yield fibrous white compound A (5.5 g).

Compound A: $[\alpha]_D^{15}$ -36.0° (1 N NaOH, c=0.25), ORD (c=0.20, 1 N NaOH), [ψ] (nm): –40(590), –100(400), –130(350), –220(300), IR ν_{max}^{KBr} cm^{-1}: 1735, 1640, 1250, 1070, 1030, 960, 900, 870, 810, 800, 770. No nitrogen content was found by elemental analysis.

Saponification: a) Compound A (40 mg) was saponified with 4% NaOH by heating for 30 minutes. The reaction mixture was neutralized with diluted HCl, and dialyzed against running water over night. The solution was concentrated under reduced pressure and filtrated to give compound B, $[\alpha]_D^{15}$ 0° (c=0.1, H_2O), IR ν_{max}^{KBr} cm^{-1}: 3300, 1150, 1000, 900, 870, 770. – b) Compound A (1 g) was saponified with 4% NaOH (30 ml) for 5 hr at room temperature. The filtrate was adjusted with diluted HCl at pH 8.0 and was evaporated to dryness. The residue dissolved in H_2O (5 ml) was acidified with 2 N HCl and refluxed with *p*-phenylphenacyl bromide (100 mg) in EtOH (10 ml) for 1.5 hr to yield *p*-phenylphenacyl acetate, mp 114–115°, Mass spectrum Calcd, for (M^+) $C_{16}H_{14}O_3$ 254.094 Found: 254.093. The IR spectrum was superimposed to that of an authentic sample.

Hydrolysis: Compound B (100 mg) was hydrolyzed with 5% H_2SO_4 (60 ml) for 6 hr by ting. The acidic solution was neutralized with barium carbonate and the filtrate was evapor- l in vacuo. The residue was dissolved in EtOH and subjected to paper partition chromato- phy to give D-mannose.

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Address: Dr. Akira Yagi, Faculty of Pharmaceutical Sciences, Kyushu University Maidashi, Higashi-ku, Fukuoka, Japan

CORRELATION BETWEEN MACROMUTANTS AND SOLASODINE CONTENT IN *SOLANUM VIARUM*

By V. R. DNYANSAGAR and A. R. PINGLE

Abstract

The seeds of Solanum viarum were treated with 0.5% EMS for 4 and 8 hours as well as with gamma rays from 10 to 40 kR to obtain mutants with higher solasodine content. The macromutants from R_2 and M_2 generations showing variability in height, branching, leaf size and weight, number of berries and their size were studied comparatively in respect of solasodine content. It is concluded that the solasodine content is directly related to the total quantity of photosynthetic tissue in the plant. A mutant containing as high as 3% solasodine is recommended for large scale cultivation.

The corticosteroids and testosteroids are abundantly used by pharmaceutical industries. They are found to be effective in the treatment of acute stages of rheumatoid arthritis, Addisson's disease, chronic cases of asthma, leukaemia, obesity, palsy, psoriasis and certain other skin diseases. India imports steroidal hormones and a continuous search is being made at present for a suitable indigenous raw material. The use of the bile acid from animals and diosgenin from *Dioscorea prezeri* and *D. deltoidea* was found economically unfeasible. Therefore, solasodine has attracted wide attention being a nitrogen analogue of diosgenin and it may be used as an intermediate for the manufacture of progesterone, cortisone and hydrocortisone. Solasodine is the aglycone of gluco-alkaloids viz. solasonine (solacarpine, purapurine), solasodamine and solamargine.

Recently *Solanum viarum* DUNAL syn. *S. khasianum* var. *chatterjeeanum* SEN GUPTA has aquired great industrial importance due to the presence of a very high concentration of gluco-alkaloids especially in the berries of the plant. It is a wild plant commonly found at Khasi, Jentia and Naga hills, Manipur and Arunachal Pradesh in the east, Dehradun forest in the north-west and Nilgiri hills in the South India. However, the solasodine content from the wildly growing plants is not sufficiently high so as to exploit the plant on commercial basis. Ionizing radiations like X-rays and gamma rays as well as chemical mutagens have been very widely used in inducing artificial mutants in numerous economically important plants. The main object of the present investigation is to find out, if there is any correlation between solasodine content and macromutants obtained after mutagenic treatments and ultimately to select a macromutant with high solasodine content.