Clinical abstracts

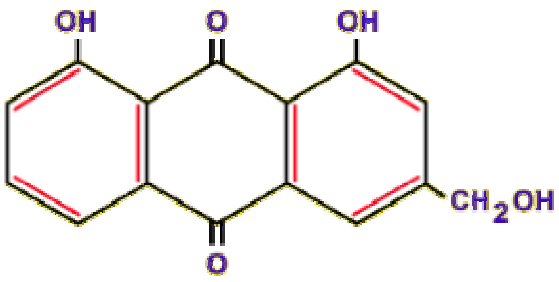
Cancer, tumors HIV & Aids

Tumor Inhibitors 114 Aloe Emodin: Antileukemic Principle Isolated From Rhamnus Frangula L
Kupchan SM; Karim A
Lloydia 39(4):223-4 1976 Jul-Aug

A systematic fractionation of an ethanol-water (1:1) extract of the seeds of Rhamnus frangula L., guided by assays for tumor-inhibitory activity, led to the isolation of Aloe emodin. This compound was found to show significant antileukemic activity against the P-388 lymphocytic leukemia in mice. A noteworthy vehicle-dependence of the testing results is reported. In the light of this vehicle-dependence, the re-examination of other anthraquinone derivatives is recommended.

Rhamnus frangula L. (Rhamnaceae) has been used in England and the United States to treat cancers, and other Rhamnus species have been used similarly in folk medicine from at least the time of Galen (circa A.D. 150) (2).

In the course of our continuing search for tumor inhibitors of plant origin, an ethanol-water (1:1) extract of the seeds of Rhamnus frangula L.[2] showed significant inhibitory activity when tested in mice against the P-388 lymphocytic leukemia[3]. Fractionation of the extract, guided by assay against the P-388 system, revealed that the inhibitory activity was concentrated in the aqueous layer of a petroleum ether-water partition, and that the active material was extractable by chloroform from the aqueous solution. Column chromatography of the chloroform solubles on SilicAR CC-7 with 2.5% methanol in chloroform led to the isolation of Aloe emodin (1) from the active chromatographic fraction. The compound was characterized by direct comparison of its melting point, tlc, and infrared spectral characteristics with those of an authentic sample of Aloe emodin.



Aloe emodin (1) shows significant inhibitory activity against the P-388 leukemia in mice when administered as a suspension in acetone-Tween 80. Results corresponding to T/C values of 133-154% were found at the optimal dose of 20 mg/kg.

In a recent review, the results of antitumor assays of 379 anthraquinone derivatives were reported. The authors concluded that "the most noteworthy observation concerning the anthraquinones is the relative lack of activity among the numerous derivatives tested from this group" (4). None were found to inhibit the L-1210 leukemia in mice, and only five showed some activity against solid tumor systems. Aloe emodin (NSC-38628) was among the derivatives which were found to be inactive. Since the P-388 system did not number among the tumors used in the study, our discovery of the antileukemic activity of Aloe emodin may reflect only a unique sensitivity of this mouse leukemia toward the compound. We note here, however, that the antileukemic activity of Aloe emodin is particularly vehicle-dependent, and that the reproducible inhibitory activity toward the P-388 system was manifested only when the acetone-Tween 80 suspension was used. In view of this fact, a re-examination of other anthraquinones for potential antitumor activity, with particular attention to possible vehicle-dependence, may be rewarded by the discovery of new and useful structure-activity relationships.

Experimental

Extraction & Fractionation -

Ground, dried seeds of Rhamnus frangula L. (1 kg) were extracted with ethanol-water (1:1, 7 liters) at room temperature overnight. The extract was filtered, concentrated under reduced pressure to about 1.5 liters and freeze-dried, to yield 163 g of residue. The residue was partitioned between petroleum ether (2 liters) and water (2 liters), whereupon 13.5 g of solid remained undissolved and was separated by filtration. Evaporation of the petroleum ether to dryness under reduced pressure yielded 11 g of residue. The aqueous solution was extracted with chloroform (2 X 2 liters), and evaporation of the chloroform extract to dryness under reduced pressure yielded 9.5 g of residue (fraction A).

Chromatography Of Fraction A -

A solution of fraction A (8 g) was treated with 25 g of SilicAR CC-7. The suspension was evaporated to dryness on a rotary evaporator, and the residue was added to a column of SilicAR CC-7 (500 g) prepared as a suspension in chloroform. The column was eluted first with chloroform (1 liter) and then with 2.5% methanol in chloroform, and 30 X 100 ml subfractions were collected. Subfractions were examined by tlc and those which were similar were combined and submitted for biological testing. The aggregate of subtractions 17-25, all rich in Aloe emodin (R_F 0.54), constituted the sole active fraction (B, 1.9 g).

Isolation Of Aloe Emodin (1) -

Active fraction B (1.5 g) was crystallized from chloroform-methanol, and recrystallization from the same solvents yielded orange-yellow needles (700 mg), mp 223-224°; lit. mp 223-225° (5). The melting point was not depressed by admixture of an authentic sample of Aloe emodin[5]. Mixture tlc and infrared spectral comparisons confirmed the identity of the twosamples.

Acknowledgments

This work was supported by grants from the National Cancer Institute (CA-11718) and the American Cancer Society (CI-102), and a contract with the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare (NO1-CM-12099). The excellent technical assistance of Mrs. C. Marcks is gratefully acknowledged.

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[1] Part 113 is reference 1.

[2] Seeds of Rhamnus frangula L. were collected in Austria in November, 1966. We acknowledge with thanks receipt of the dried plant material from Dr. R. E. Perdue, Jr., U.S. Department of Agriculture, in accordance with the program developed by the National Cancer Institute. Voucher specimens are on deposit at the Medicinal Plant Resources Laboratory, Agricultural Research Service, Beltsville, Maryland.

[3] Antileukemic activity was assayed under the auspices of the National Cancer Institute, by the procedure described in reference 3.

[4] Melting points were determined with a Mettler FP2 hot-stage microscope. Infrared spectra were determined with a Perkin-Elmer Hitachi model 257 spectrophotometer as KBr pellets. Petroleum ether refers to the fraction of bp 60-68°. Thin-layer chromatography was carried out on silica gel 60 F-254 (E. Merck) precoated plates, and chromatograms were visualized by spraying with an anisaldehyde-sulfuric acid spray; the developing solvent was 5% methanol in chloroform.

[5] We thank Professor H. Wagner, Universität München, for an authentic sample of Aloe emodin

Cancer Research

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Texas A&M

Research by the immunologist Ian Tizard, Ph.D. and virologist Maurice Kemp, Ph.D. from Texas A&M led to the discovery that Aloe mucopolysaccharide is taken into a special leukocyte, the macrophage, and this cell is stimulated to release messenger molecules called cytokines (interferons, interleukines, prostaglandins, tumor necrosis factor and stem-cell growth factors.) Tumors release a chemical that attracts blood circulation so that malignant cells have a supply to the tumor and it therefore dies. All of the immune modulating effects from Aloe contribute greatly to the prevention and healing of malignant cells.

Anticancer Effects Of Aloe On Sarcoma 180 In ICR Mouse & On Human Cancer Cell Lines

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Coll. Pharm., Sung Kyun Kwan Univ.
Yakhak Hoeji 38 (3). 1994. 311-321

Anticancer effects of Aloe on sarcoma 180 in ICR mouse or human cancer cells were determined. Sarcoma 180 cells were inoculated subcutaneously into male ICR mouse to determine effect of Aloe on tumor growth, or inoculated intraperitoneally into male ICR mouse to determine effect of Aloe on life span prolongation, followed by oral administration of Aloe vera (10 mg/kg/day, 50 mg/kg/day) or Aloe arborescens (10 mg/kg/day, 100 mg/kg/day) once a day for 14 days. The administration of Aloe vera or Aloe arborescens did not suppress tumor growth. However the life span of ICR mouse was prolonged to 19% (P lt 0.05), 22% (P lt 0.05), and 32% (P lt 0.05) by administration of Aloe vera 10 mg/kg/day, Aloe vera 50 mg/kd/day, and Aloe arborescens 100 mg/kg/day, respectively. To determine anticancer effect of Aloe in vitro, Aloe extract was added to the culture of human gastric cancer cells (SNU-1) and colorectal cancer cells (SNU-C2A), and concentration of Aloe to inhibit cancer cell growth was determined using MTT (3 - (4, 5-dimethylthiazol-2-yl) -2, 5-diphenyltetrazolium bromide) cytotoxicity assay. High ID-50 values of Aloe vera and Aloe arborescens against gastric cancer cell line (SNU-1) and colorectal cancer cell line (SNU-C2A) suggest that Aloe gel does not have anticancer effect on these specific human cancer cells although high concentration of Aloe inhibited growth of human cancer cells significantly.

Antimutagen Of Aloe Plants

Nakasugi, Tohru; Komai, Koichiro
Res. Lab. Med, Prod. Plant Origin
Kinki Daigaku Nogakubu Kiyo (1994), 27, 47-54

An antimutagen from Aloe Arborescens Mill was isolated and identified. Methanol exts. from dried leaves of A. arborescens inhibited frameshift mutation induced by 3-amino-1-methyl-5H-pyrido [4, 3b] indole in Salmonella typhimurium TA98. The antimutagen isolated from the methanol exts. was identified as the anthraquinone Aloe-emodin. Aloe-emodin

inhibited frameshift mutation by 60.3% at 0.1 mM/plate and 86.3% at 1.0 mM/plate whereas barbaloin, monoglucoside of Aloe-emodin, did not. Fresh A. aborescens leaves contained 1.17 ug/g (wet wt.) of Aloe-emodin. Aloe-emodin was also detected in A. ferox, A. vera, A. eru, and A. compacta by HPLC. These Aloe species may have substances that are useful for prevention of some forms of cancer.

Aloemannan, Significant Antitumor Efficacy

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Health Science Center, University Of Texas

In 1977 while conducting a series of animal experiments using aloemannan a mucopolysaccharide of Aloe arborescens, detected aloemannan a significant antitumor efficacy. Unlike usual anticancer drugs killing cancer cells directly, it acts as a stimulus for the body's defense mechanism, or immunity to suppress tumor. In other words, it prohibits multiplication of cancer cells while it is coexistent with them. Prof. Winters and his group of the Health Science Center at the University of Texas verified their test-tube experiments using human cervical cancer cells that Aloe vera extract prohibits the growth of cancer cells.

The Preventive & Therapeutic Potential Of The Squalene-Containing Compound Roidex, On Tumor Promotion & Regression

Desai KN; Wei H; Lamartiniere CA
Department Of Pharmacology & Toxicology, University Of Alabama
Cancer Letters 101(1):93-6 1996 Mar 19

Recent scientific evidence has shown free radicals or reactive oxygen species (ROS) to play an important role in the initiation and progression of cancer. Many radical scavengers have also been found to help reduce the attacks by these ROS. Interestingly, the ROS scavengers that have been investigated are naturally occurring compounds such as vitamins C and E. Roidex is a formulation of squalene, vitamin E, and Aloe vera. It was our goal to investigate whether Roidex was able to prevent the development of chemically induced cancer and to cause regression of any tumors already formed in a mouse skin model. In the prevention study, skin tumors were initiated in 50 female CD-1 mice with 7,12-dimethylbenz[a]-anthracene (DMBA) and promoted with 12-O-tetradecanoylphorbol-13-acetate (TPA). The mice were treated with either mineral oil, 5% squalene, or Roidex. At the end of the prevention study, there was a 33.34% incidence to tumors (multiplicity of 1.40) in the mineral oil-treatment group, 26.67% (multiplicity of 0.467) in the 5% squalene and Roidex groups, respectively. The tumor regression study involved the selection of mice with tumors and possible regression of these tumors with Roidex treatment. There was a regression of 33.34% of the tumors in the Roidex-treated group (39 tumors to 26 tumors) compared to the non-treated group whose tumors regressed only 3.44% (29 tumors to 28 tumors).

Plant Lectin, ATF1011, On The Tumor Cell Surface Augments Tumor-Specific Immunity Through Activation Of T Cells Specific For The Lectin

Yoshimoto R; Kondoh N; Isawa M; Hamuro J

Cancer Immunol Immunother 25(1):25-30 1987

The possibility that a plant lectin as a carrier protein would specifically activate T cells, resulting in the augmentation of anti-tumor immunity was investigated. ATF1011, a nonmitogenic lectin for T cells purified from Aloe arborescens Mill, bound equally to normal and tumor cells. ATF1011 binding on the MM102 tumor cell surfaces augmented anti-trinitrophenyl (TNP) antibody production of murine splenocytes when the mice were primarily immunized with TNP-conjugated MM102 tumor cells. The alloreactive cytotoxic T cell response was also augmented by allostimulator cells binding ATF1011 on the cell surfaces. These augmented responses may be assumed to be mediated by the activation of helper T cells recognizing ATF1011 as a carrier protein. Killer T cells were induced against ATF1011 antigen in the H-2 restricted manner using syngeneic stimulator cells bearing ATF1011 on the cell surfaces. When this lectin was administered intralesionally into the tumors, induction of cytotoxic effector cells was demonstrated. These results suggest that intralesionally administered ATF1011 binds to the tumor cell membrane and activates T cells specific for this carrier lectin in situ, which results in the augmented induction of systemic anti-tumor immunity.

Antimetastatic Properties Of Aloe Juice

Gribel' NV; Pashinskii VG

Vopr Onkol 32(12):38-40 1986

An evaluation of antimetastatic properties of succus Aloes was carried out using three types of experimental tumors of mice and rats. It was found that succus Aloes treatment contributes to reduction of tumor mass, metastatic foci and metastasis frequency at different stages of tumor progress without affecting major tumor growth. Succus Aloes potentiates the anti-tumor effect of 5-fluorouracil and cyclophosphamide as components of combination chemotherapy.

Effects Of Aloe Extracts On Human Normal & Tumor Cells In-Vitro

Winters WD; Benavides R; Clouse WJ
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Econ Bot 35 (1), 1981, 89-95

Fractions of leaf extracts from 2 local types, labeled Aloe vera (subsequently identified as A. barbadensis Mill., and A. saponaria Haw.), were prepared by differential centrifugation and tested by in vitro assays for the presence of lectin-like activities and for effects on the attachment and growth of human normal and tumor cells. Fractions of extracts of fresh leaves and commercially A. vera gel had high levels of lectin-like substances measured by immunodiffusion and nemagglutination assays. Substances in fluid fractions from both fresh leaf sources markedly promoted attachment and growth of human normal cells.

Epidemiologic Survey On Lung Cancer With Respect To Cigarette Smoking & Plant Diet

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Jpn J Cancer Res 80(6):513-20 1989 Jun

This case-control study of lung cancer was based on a cross-sectional questionnaire survey of inpatients at 5 general hospitals in Okinawa, Japan, from 1982 to 1987. The purpose of the study was to clarify the relations of lung cancer to cigarette smoking and plant diet. Ingestion frequencies of 17 major dietary plants and/or herbs were obtained by means of a questionnaire interview. As eligible subjects for a case-control analysis, there were 673 respondents aged over 30 years with clear smoking history, age, sex and diagnosis. Psychiatric patients were excluded. Odds ratios of newly diagnosed lung cancer were calculated by the Mantel-Haenszel procedure. A pair consisted of a case and two controls which were selected randomly by using multivariate caliper matching. Sixty-four pairs matched for age (+/- 5) and sex showed a significantly high odds ratio of 2.9 (P less than 0.0005). However, three male groups who were categorized by the number of cigarettes smoked did not exhibit dose-dependency of lung cancer on smoking. Lung cancer was more prevalent in exsmokers than in current smokers. Case-control analyses by male generations revealed that lung cancer incidence was age-dependent, and there was a clear dose-response relationship between smoking and lung cancer in males in their sixties. A case-control analysis of each of 17 edible plants based on 44 pairs who were matched for age (+/- 5), sex and smoking history demonstrated that the odds ratio of Aloe (Aloe arborescens Mill var. natalensis Berger) was 0.5 (P less than 0.1), suggesting that the Aloe may prevent human carcinogenesis at various sites.

Aloe Vera & AIDS Research

Various AIDS studies were completed by researchers such as Dr. Terry Pulse, M.D., Dr. Reg McDaniel, M.D., Dr. Terry Watson, D.O., Dr. Clumeck, M.D. (of Belgium) and others throughout the 1980's using oral mucopolysaccharides. The results were impressive, demonstrating in many of the studies an average of 70% improvement in symptoms and laboratory criteria within 3 to 4 months. Many patients stated that opportunistic infections had stopped and they were able to return to normal activity. In one dramatic case, a man with advanced AIDS had 17 liver tumors and after one and a half years on oral Aloe mucopolysaccharides, his T-Cell count was normal and all the tumors had disolved (confirmed by x-ray films).

Lab studies showed that helper lymphocytes (CD4) rose to three times the pre-treatment levels. HIV-1 virus could no longer be cultured. P-24 antigen levels for the virus dropped or became negative.

Researchers at Vanderbilt Medical Center in Nashville, Tennessee discovered that Aloe mucopolysaccharides alters synthesis and thus the structure of the AIDS virus envelope necessary for infecting lymphocytes. Further studies at The Southern Research Institute found that there is suppression of the viral messenger RNA in HIV-1 infected leukocytes. Therefore, the reproduction of HIV-1 is inhibited with a natural and non-toxic substance.

In studies completed at the Fort Worth Medical Center Complex it was demonstrated that a person's leukocytes were rendered resistant to HIV-1 virus in culture tests outside the body.

A Study By Dr. Terry Pulse, M.D. Of 29 AIDS Patients

Journal of Advancement in Medicine Winter 1990, Volume 3, No. 4

Patients took 1200 mg of the active ingredient in Aloe vera juice daily as well as nutrient supplements. We quote directly from Dr. Pulse's report of the results, which are fantastic: "No adverse effects attributable to the essential fatty acid capsules were observed nor any side effects of the nutritional supplementation powder nor of the Aloe vera juice. Most patients who were symptomatic reported that within three to five days their energy levels improved, fever disappeared, night sweats stopped, cough decreased or stopped altogether, shortness of breath decreased, lymph nodes decreased in size, diarrhea stopped, strength improved and the only measurable side effect of this particular study was weight gain, which is a desirable effect. There were no biochemical abnormalities noted on MAC in this particular study. AZT induced anemia improved on this particular regimen. Chest x-rays remained normal throughout the study. No changes in EKG from baseline were observed. There was great improvement in all patients to hypersensitivity skin testing at the end of 90 days... Not only did the patients improve clinically and functionally, but their Karnofsky scores improved in 93.1% of the patients at 90 days and in 100% at 180 days. 51.7% of the patient's T4 helper lymphocytes increased at 90 days and 32.2% at 180 days, with 25% reactive HIV P24 core antigen converted to negative at 90 days and 180 days."

In essence, a substantial number of patient's physical condition improved. Energy levels improved, fever disappeared, night sweats stopped, cough decreased or stopped, shortness of breath decreased, lymph nodes decreased in size, diarrhea stopped, weakness improved. Hypersensitivity skin testing improved. In 96.4% of the test patients, their Modified Walter Reed Scores had improved at 180 days. Karnofsky scores improved in 93.1%. T4 lymphocytes increased in some patients and, in some, their reactive HIV P24 antigen converted to negative.

Substance Boosts Therapeutic Effects Of AZT

Texas A & M University

Aids Weekly August 5, 1991 p2(2)

A team of scientists from Texas A&M University, College Station, and three other institutions says that a complex carbohydrate compound purified from the Aloe vera plant appears to help drugs such as azidothymidine (AZT) and acyclovir (ACY) block the pathology associated with HIV and herpes simplex virus (HSV). They also found that the compound interfered with HIV's ability to reproduce in infected cells. "It's not going to be a magic bullet against AIDS," cautions Dr. Maurice C. Kemp, a virologist in Texas A&M's College of Veterinary Medicine. "There aren't many magic bullets out there. But as an adjunctive therapy, it looks like it can be used in combination with other therapies." The scientists' findings are published in the July, 1991, issue of the new *Journal Molecular Biotherapy* with additional results scheduled to be published in the September, 1991, issue.

Aloe Vera May Mimic AZT Without Toxicity

McDaniel, H Reginald

Medical World News December 1993

A preliminary study suggests that the Aloe vera may mimic AZT without toxicity. A substance in Aloe vera show signs of boosting the immune systems of AIDS patients and blocking the human immune-deficiency virus spread without the toxic side effects.

Mechanisms Of Ultraviolet Induced Immune Suppression

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Crisp Data Base National Institutes Of Health

RPROJ/CRISP Epicutaneous exposer to ultraviolet (UV) radiation suppresses T cell-mediated immune responses to antigens encountered in the skin and permits the growth of highly immunogenic skin cancers in laboratory animals. Immune suppression by UV radiation is mediated by multiple, complex, and interacting mechanisms. Recent studies indicate the suppression is triggered by DNA damage followed by production of immunosuppressive cytokines, loss of antigen presenting cells (APC) from the skin, alteration of the functions of remaining APC, and induction of antigen-specific suppressor T cells. However, the regulation and interaction of these APC, and induction of antigen-specific suppressor T cells. However, the regulation and interaction of these APC and cytokine pathways are unclear and appears to be different for contact hypersensitivity (CHS) reactions to allergens in skin and delayed type hypersensitivity (DTH) reactions to micro-organisms. We have recently shown that crude extracts of Aloe barbadensis gel protects CHS and DTH responses against suppression by UV radiation. Because Aloe extract provides broad protection for immune responses that are abrogated by UN by different mechanisms, it may act as a central controlling point in suppression. Alternatively Aloe may contain several agents that act on CHS and DTH separately. Furthermore, Aloe is chemically distinct from antibodies, cytokines, or other agents that have been used to probe suppression pathway induced by UV radiation, and it may be acting by a novel mechanism(s). We will test these hypotheses by exposing mice to UV radiation and examining the effect of Aloe treatment on the production of the regulatory cytokines TNF-alpha, IL-10, and Il-12 in cultured keratinocyte cell liens and in skin. We will investigate whether protection of CHS and DTH responses is mediated separately by different components in Aloe. We will also examine the effect of Aloe on the function of APC from the draining lymph nodes using the murine model of CHS response to the hapten fluorescein isothiocyanate, and the DTH response to Candida albicans. In addition, we propose to investigate the ability of Aloe to preserve immunity to UV-induced skin cancers. Clarification of the relationship of the CHS and DTH models to cutaneous tumor immunity may permit the design of therapeutic agents that are more effective in protecting humans against the development of skin cancer.

Immunomodulating Properties Of An Extract Isolated & Partially Purified From Aloe Vahombe Study Of Antitumoral Properties & Contribution To The Chemical Nature & Active Principle

Ralamboranto L; Rakotovao LH; Le Deaut JY; Chaussoux D; Salomon JC; Fournet B; Montreuil J; Rakotonirina-Randriambeloma PJ; Dulat C; Coulanges P

Arch Inst Pasteur Madagascar 50(1):227-56 1982

An immuno-modulator fraction (Alva) extracted from an endemic plant, in the south of Madagascar, the Aloe vahombe, significantly protects mice against bacterial, parasitic and fungal infections. Wishing to verify whether the fraction Alva was active in tumour reduction, we studied its effect on the development of experimental fibrosarcoma and melanoma in mice by intravenous and intracutaneous injections and injections directly into the tumor of the immunostimulant fraction. We have observed cures, only in the case of the McC3-1 tumor but it is encouraging to note that under different experimental conditions the rate of growth of tumors in animals which were treated is slower than in those not treated. The Alva fraction is a substance which is hydrosoluble, thermostabile, having a molecular weight exceeding 30,000 and is a polysaccharide. The predominant sugars are glucose and mannose in 3:1 ratio. Preliminary studies of its action seem to indicate that the Alva fraction acts upon nonspecific response and could possibly stimulate the phagocyte activity of the peritoneal macrophagus.

Purification Of Active Substances Of Aloe Arborescens Miller & Their Biological & Pharmacological Activity

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Phytother Res. (1993) 7 (Spec. Issue, Proceedings of the International Congress of Phytotherapy, 1991), S14-S19

The authors purified Aloctin A from Aloe arborescens Miller and defined its chem., biol. and pharmacol. activities. Aloctin A consists of two discrete bands, a and b with a combined S-S bond. Its mol. wt. for a is 7500 and the mol. wt. for b is 10,500. Aloctin A has many biol. and pharmacol. activities as follows: 1. hemagglutinating activity; 2. cytoagglutinating activity; 3. mitogenic activity of lymphocytes; 4. ppt. - forming reactivity with a2-macroglobulin; 5. complement C3 activating activity; 6. inhibition of heat-induced hemolysis of rat erythrocytes; 7. anti-tumor effect; 8. anti-inflammatory effect; 9. inhibition of gastric secretion and gastric lesions.
