The Reporter

Reprinted from Volume 14, No. 5, 1995

T295015

© 1999 Sigma-Aldrich Co.

For more information, or current prices, contact your nearest Supelco subsidiary listed below. To obtain further contact information, visit our website (www.sigma-aldrich.com), see the Supelco catalog, or contact Supelco, Bellefonte, PA 16823-0048 USA.

ARGENTINA Ž Sigma-Aldrich de Argentina, S.A. • Av. Pueyrredon 2446/50 • Piso 5-B • Buenos Aires 1119
AUSTRALIA • Sigma-Aldrich Pty. Ltd. • Unit #2, 14 Anella Avenue • Castle Hill NSW 2154
AUSTRIA • Sigma-Aldrich Handels GmbH • Hebbelplatz 7 • A-1110 Wien
BELGIUM • Sigma-Aldrich N.V./S.A. • K. Cardijplein 8 • B-2880 Bornem
BRAZIL • Sigma-Aldrich Quimica Brasil Ltda. • Rua Sabara, 566-Conj. 53 • 01239-010 São Paulo, SP
CANADA • Sigma-Aldrich Canada, Ltd. • 2149 Winston Park Dr., Oakville, ON L6H 6J8
CZECH REPUBLIC • Sigma-Aldrich s.r.o. • Pobrezni 46 • 186 21 Praha 8
DENMARK • Sigma-Aldrich Denmark A/S • Vejlegaardsvej 65B • DK-2665 Vallensbaek Strand
FINLAND • Sigma-Aldrich Finland/YA-Kemia Oy • Teerisuonkuja 4 • FIN-00700 Helsinki
FRANCE • Sigma-Aldrich Chimie • Chromatographie Supelco • L'Isle d'Abeau Chesnes - B.P. 701 • 38297 Saint-Quentin Fallavier Cedex
GERMANY • Sigma-Aldrich Chemie GmbH • Geschäftsbereich Supelco • Grünwalder Weg 30 • D-82041 Deisenhofen
GREECE • Sigma-Aldrich (o.m.) Ltd. • 72 Argonafton Str. • 16346 Ilioupoli, Athens
HUNGARY • Sigma-Aldrich Kft. • Nagy Diófa u. 7., IV fl. • H-1067 Budapest
INDIA • Sigma-Aldrich Co. • Survey No. 31/1, Sitharamapalaya • Mahadevapura P.O. • Bangalore 560 048
IRELAND • Sigma-Aldrich Ireland Ltd. • Airton Road • Tallaght • Dublin 24
ISRAEL • Sigma Israel Chemicals Ltd. • Park Rabin • Rohovot 76100
ITALY • Sigma-Aldrich s.r.l. • Via Gallarate, 154 • 20151 Milano
JAPAN • Sigma-Aldrich Japan K.K. • Division Supelco • JL. Nihonbashi Building • 1-10-15 Nihonbashi Horidome-cho, Chuo-ku • Tokyo 103
KOREA • Sigma-Aldrich Korea • Samhan Camus Annex, 10th Floor • 17-26 Yoido-dong, Yungdeungpo-ku • Seoul
MALAYSIA • Sigma-Aldrich (M) Sdn. Bhd. • 9-2, Jalan 2/128, Taman Gembira • Off Jalan Kuchai Lama • 58200 Kuala Lumpur • Selangor
MEXICO • Sigma-Aldrich Química S.A. de C.V. • Calle 6 North No. 107 • Parque Industrial Toluca 2000 • 50200 Toluca
NETHERLANDS • Sigma-Aldrich Chemie BV • Postbus 27 • 3330 AA Zwijndrecht
NORWAY • Sigma-Aldrich Norway • Sandakerveien 102 • N-0483 Oslo
POLAND • Sigma-Aldrich Sp. z o.o. • Szelagowska 30 • 61-626 Poznañ
PORTUGAL • Sigma-Aldrich Quimica, S.A. • P.O. Box 131 • Sintra 2710
RUSSIA • Sigma-Aldrich Russia • TOO Techmedbiochem • Makarenko Str. 2/21 • Building 1, Flat 22 • Moscow 103062
SINGAPORE • Sigma-Aldrich Pte. Ltd. • 102E Pasir Panjang Road • #08-01 Citilink Warehouse • Singapore 118529
SOUTH AFRICA • Sigma-Aldrich (pty) Ltd. • CNR Kelly & Ackerman Streets • Southern Life Industrial Park Unit • Unit 16/17 • Jet Park 1459
SPAIN • Sigma-Aldrich Quimica, S.A. • Apt. Correos 161 • 28100 Alcobendas, Madrid
SWEDEN • Sigma-Aldrich Sweden AB • Solkraftsvägen 14C •135 70 Stockholm
SWITZERLAND • Supelco Switzerland • Industriestrasse 25 • P.O. Box 260 • CH-9471 Buchs
UNITED KINGDOM • Sigma-Aldrich Company Ltd. • Supelco UK • Fancy Road, Poole • Dorset BH12 4QH
UNITED STATES • Supelco • Supelco Park • Bellefonte, PA 16823-0048 · Phone 800-247-6628 or 814-359-3441 · Fax 800-447-3044 or 814-359-3044 · email:supelco@sial.com

H

This article is archived from a past issue of The Supelco Reporter. Information in the article was appropriate at the time of publication, but product specifications, catalog numbers, and availability may have changed over time.

If you have questions about applying methodology described in this article to a current application, please contact our technical service chemists.

ISO 9001 REGISTERED

Supelco is a member of the Sigma-Aldrich family. Supelco products are sold through Sigma-Aldrich, Inc. Sigma-Aldrich warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product for a particular use. Additional terms and conditions may apply. Please see the reverse side of the invoice or packing slip.

Anti-Inflammatory Components of Mushroom Extract on SUPELCOSIL ABZ+Plus HPLC Columns

R.T. Streeper, N. Satsangi,
W.B. Stavinoha, S.T. Weintraub
(The University of Texas Health Science Center at San Antonio)

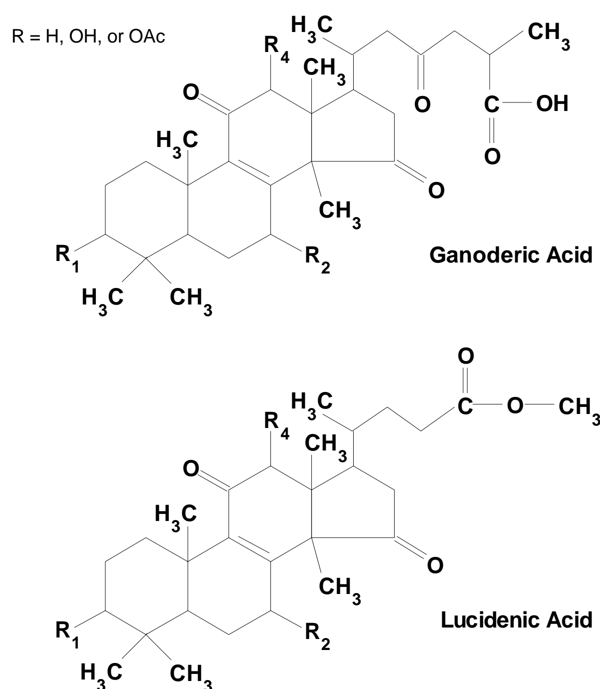
Ganoderma lucidum, a mushroom long used in the East for treating a broad range of disorders, contains numerous pharmacologically active molecules, including an exceptionally diverse family of oxygenated triterpenes. The authors of this contributed article have determined that extracts of G. lucidum possess potent anti-inflammatory properties. They present some of their initial observations on the biological activity and chromatography of these acids. Standard reversed-phase fractionation does not resolve the hundreds of structural variants of ganoderic and lucidenic acids produced by the mushroom. However, semi-preparative C18 chromatography followed by sub-fractionation on a SUPELCOSIL ABZ+Plus column separates, to a significant degree, many of the acids.

Anti-inflammatory agents currently on the market have serious side effects that significantly limit their usefulness. For example, gastropathy caused by aspirin-like drugs accounts for at least 70,000 hospitalizations in the U.S. each year, and an estimated 7,000 to 20,000 deaths. Furthermore, aspirin-like compounds provide only symptomatic relief and do not treat underlying pathology. Steroids, on the other hand, frequently cause suppression of pituitary-adrenal function and seriously disturb fluid and electrolyte balance. In children, steroid use can prevent growth and can even cause death.

Ganoderma lucidum and preparations of this mushroom have a long history of use throughout the East as traditional medicines in the treatment of a broad range of disorders (1). More recently it has been found that this mushroom contains a number of pharmacologically active molecules: anti-tumor, hypoglycemic, and anti-fibrotic polysaccharides (2-4), an immunomodulary protein (5), chemoprotective principles (6), a unique inhibitor of platelet aggregation (7), and an exceptionally diverse family of oxygenated triterpenes that is comprised predominantly of ganoderic and lucidenic acids (8) (Figure A).

Amelioration of the pain and inflammation associated with arthritis has historically been attributed to preparations of *G. lucidum* (9). We have determined that extracts rich in ganoderic and lucidenic acids, prepared from various portions of *G. lucidum,* possess potent systemic and topical anti-inflammatory properties (10). In this report, we present some of our initial observations on the biological activity of *G. lucidum* extracts, as well as the results of preliminary chromatographic analysis.

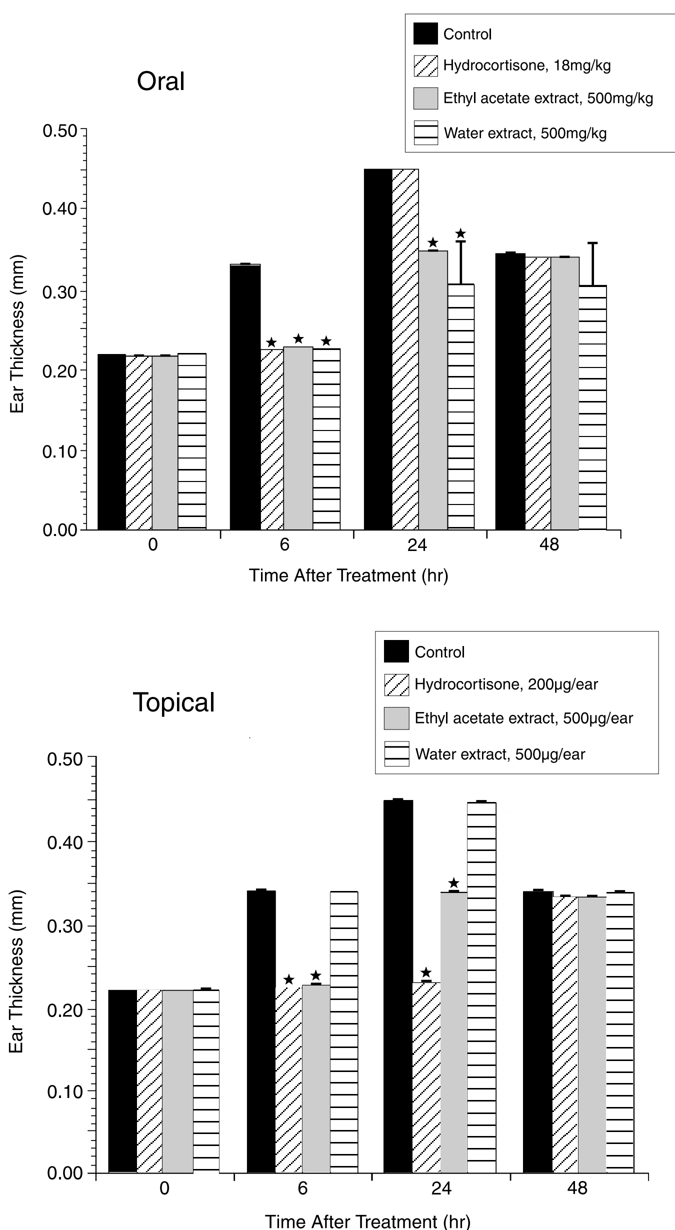
Figure A. Ganoderic and Lucidenic Acids



Electrospray ionizaton mass spectra were acquired on a Finnigan-MAT SSQ700 quadrupole mass spectrometer fitted with an Analytica of Branford electrospray interface. Samples were introduced in 70% aqueous acetonitrile/0.5% acetic acid solution at a flow rate of 1µL/min, with an electrospray voltage of -3.5kV.

Semi-preparative-scale liquid chromatographic fractionations were performed with a Milton Roy ternary gradient HPLC system, using a 10 x 250mm octadecyl column. A non-linear stepped gradient consisting of water/acetonitrile/5% aqueous acetic acid running 60 minutes at 2mL/min was used to effect separations; 1-min fractions were collected from 9 to 55 minutes. The fractions were lyophilized and resuspended in 70% aqueous acetonitrile for further chromatographic and mass spectrometric analyses. The fractions were sub-fractionated on an analytical scale using a Waters HPLC system with a 4.6 x 250mm SUPELCOSIL™ ABZ+Plus mixed functionality column and a nonlinear binary gradient

Figure B. Anti-Inflammatory Activity of Extracts of *Ganoderma lucidum* Pore Powder



Animals: male Balb/C mice, 19-21g (oral admin.) or 21-25g (topical admin.), 3/group
Irritant: 25mg croton oil in 1mL pyridine/water/diethyl ether, 4:1:5; 10µL applied via Eppendorf pipette to inner side of left ear. Ear thickness measured with an Oditest caliper before application of irritant and at specific times after treatment with test solution. Values represent mean ±1 standard deviation.

Oral Administration: 1mL test solution administered by gavage 1 hr before application of irritant. Water extract dissolved in water, ethyl acetate extract suspended in 1% aqueous methyl cellulose, control group administered 1mL water. *Statistical significance of difference from control group: p<0.001.

Topical Administration: Each test material dissolved in pyridine/water/diethyl ether (4:1:5) and applied to same location as irritant, 30 min after treatment with irritant. Control group treated with irritant alone. *Statistical significance of difference from control group: p<0.0001.

consisting of 0.5% acetic acid in water/0.5% acetic acid in acetonitrile at 1mL/min for 70 minutes. In both the semi-preparative and analytical separations, column effluent absorbance was monitored at 244nm.

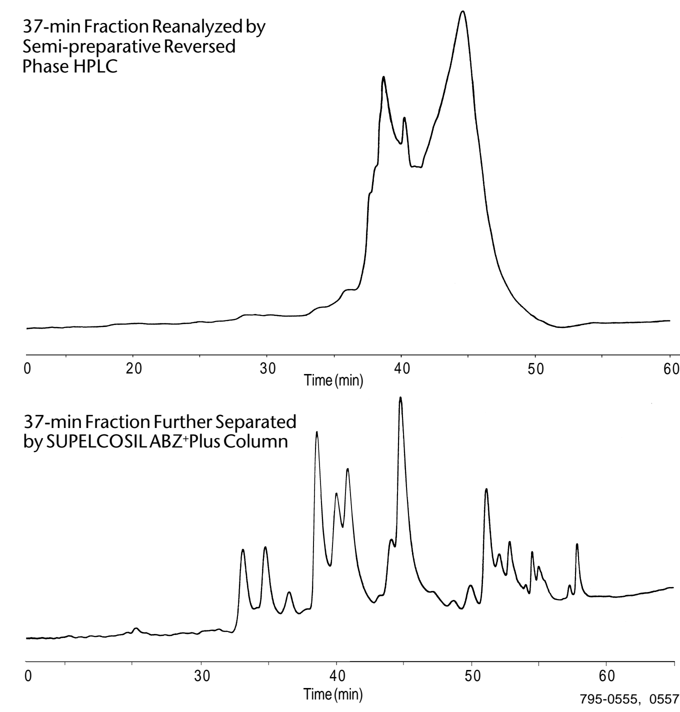
Biological Activity - In view of the tremendous structural heterogeneity of the acidic triterpenes produced by *G. lucidum* and the effort required to separate sufficient quantities of pure ganoderic acids for controlled testing of *in vivo* activity, for our preliminary investigation we assessed the anti-inflammatory efficacy of crude water and ethyl acetate extracts of the mushroom. For our *in vivo* assay, mice were treated either orally or topically with water and ethyl acetate extracts of *G. lucidum*. The ability of these extracts to alleviate the swelling produced by an irritant, croton oil, was compared to the efficacy of one of the most commonly used topical anti-inflammatory drugs, hydrocortisone. Figure B summarizes the results of these experiments.

It is clear that orally administered ethyl acetate and water extracts of *G. lucidum* have significant anti-inflammatory activity that extends over a longer period of time than does hydrocortisone. Moreover, topical application of the ethyl acetate extract results in significant anti-inflammatory activity that compares favorably with the activity of hydrocortisone. The water extract, on the other hand, has little or no topical effect.

The mice used in this study were sacrificed at the end of each experiment and the weight of the thymus in each animal was assessed. None of the animals in the irritant alone (control), water extract, and ethyl acetate extract groups exhibited a thymus weight significantly lower than normal. The animals treated with hydrocortisone, however, showed significant thymic involution, a toxicity associated with corticosteroid treatment. Thus, extracts of *G. lucidum* possess significant anti-inflammatory activity, and do not appear to exhibit the unwanted side effects encountered with corticosteroids. On the basis of these preliminary results for anti-inflammatory efficacy, we have now begun screening semi-preparative HPLC fractions for *in vivo* activity, thus allowing us to proceed more directly to the active components.

HPLC Fractionation - Analysis of the ganoderic acids of *G. lucidum* is complicated by the exceedingly large number of ganoderic and lucidenic acids produced by the mushroom. Standard separation techniques relying on C18 reversed-phase fractionation are not equal to the task of resolving the hundreds of structural variants that are present. However, if semi-preparative C18 chromatography is followed by sub-fractionation using Supelco's new mixed function ABZ+Plus column, it is possible to separate, to a significant degree, a large number of the ganoderic acids. Figure C shows an HPLC chromatogram of the 37-minute fraction collected during semi-preparative C18 separation and reinjected onto the semi-preparative column for further separation under the same conditions. Immediately apparent is the poor resolution of the components. Figure C also illustrates fractionation of the same sample by the ABZ+Plus column. Even though resolution is still not complete, we are able to resolve a much larger number of components.

Figure C. SUPELCOSIL ABZ+Plus Column Enhances Resolution of *G. lucidum* Extract



The eluate from the ABZ+Plus column was collected in 1-min fractions and analyzed by direct infusion ESI-MS. The results of these analyses confirmed that the ABZ+Plus column effected considerable separation of the components that eluted together from the C18 column. Thus, the use of two columns of differing selectivity has allowed us to partially untangle the triterpene products of *G. lucidum.*

Our data show that the extracts of *G. lucidum* possess significant anti-inflammatory activity. While it is premature at this stage of the investigation to assign a rank order of potency to all of the members of this complex mixture of acidic triterpenes, our preliminary studies suggest that relatively minor structural differences among the components lead to measurable differences in anti-inflammatory potency. As we improve our abilities to isolate and characterize individual ganoderic acids, we expect to be able to deduce comprehensive structure-activity relationships for these molecules. This effort will be important not only for elucidating the mechanism of action of these molecules, but also for future analog drug development.

Ordering Information:

SUPELCOSIL ABZ+Plus Columns

Description	Cat. No.
5cm x 4.6mm, 5µm particles	**59195-U**
15cm x 4.6mm, 5µm particles	**59196**
25cm x 4.6mm, 5µm particles	**59197**

For a complete list of SUPELCOSIL ABZ+Plus columns (microbore, preparative, modular, and guard columns), please request Product Specification 494128.

References

1. Liu, G.T., *Mushroom Biology and Mushroom Products* S.T. Chang, J.A. Buswell, and S.W. Chiu, eds., The Chinese University Press, Hong Kong, p. 267 (1993).
2. Sasaki, T., Y. Arai, T. Ikekawa, G. Chihara, and F. Fukouka, *Chem. Pharm. Bull.* (Tokyo), **19:** 821 (1971).
3. Hikuno, H. and T. Mizuno, *Planta Med.,* **55:** 385 (1989).
4. Sohn, D.H., E.J. Park, G. Ko, and J. Kim, *Proc. Sixth Int. Symp. Ganoderma lucidum,* p. 29 (April 12, 1995).
5. Kino, K., T. Sone, J. Watanabe, A. Yamashita, H. Tsuboi, H. Miyajima, and H. Tsunoo, *Int. J. Immunopharmacology,* **13:** 1109 (1991).
6. Wong, B.Y., B.H. Lau, P.P. Tadi, and R.W. Teel, *Mutat. Res.,* **279:** 209 (1992).
7. Kawagishi, H., *Proc. Sixth Int. Symp. Ganoderma lucidum* (Apr. 1995).
8. Toth, J.O., B. Luu, and G. Ourisson, *Tetrahedron Lett.,* **24:** 1081 (1983).
9. Lin, J.M., C.C. Lin, H.F. Chiu, J.J. Yang, and S.G. Lee, *Am. J. Chin. Med.,* **21:** 59 (1993).
10. Stavinoha, W.B., *Proc. Sixth Int. Symp. Ganoderma lucidum,* p. 3 (Apr. 1995).

References not available from Supelco.

Acknowledgments

The authors wish to convey their appreciation to Lone Star Growers (San Antonio, Texas) and the Il Yang Pharmaceutical Company, Ltd. (Seoul, Korea) for their support of this investigation. We are also grateful to Tracy Ascah and Supelco for generously donating the SUPELCOSIL ABZ+Plus HPLC columns, which have been instrumental in resolving the tremendously complex mixtures of ganoderic acids.

Ed. note

We have not included the authors' mass spectra, due to space constraints. Readers interested in additional details of this investigation are invited to contact the authors at Department of Biochemistry, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio,Texas 78284-7760 USA.

SUPELCOSIL is a trademark of Sigma-Aldrich Co.