Advances in BioChirality

Advances in BioChirality

edited by

G. Pályi
C. Zucchi
Department of Chemistry
University of Modena
Modena, Italy

L. Caglioti
"La Sapienza"
Roma, Italy

1999

ELSEVIER
Amsterdam – Lausanne – New York – Oxford – Shannon – Singapore – Tokyo

ELSEVIER SCIENCE Ltd
The Boulevard, Langford Lane
Kidlington, Oxford OX5 1GB, UK

First edition 1999

Library of Congress Cataloging in Publication Data
A catalog record from the Library of Congress has been applied for.

British Library Cataloguing in Publication Data
A catalogue record from the British Library has been applied for.

ISBN: 0 08 043404 5

∞ The paper used in this publication meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).

Printed in The Netherlands.

Advances in BioChirality

Editors

Gyula PÁLYI, PhD, DSc. Born 06.06.1936, Budapest (Hungary). Graduated at the Technical University of Budapest and at the University of Pisa (Italy). Professor of Organic Chemistry (1976) at the University of Veszprém (Hungary), and professor of Coordination Chemistry at the University of Modena (1987). Member of the Academy of Sciences of Bologna (1978). Author of cca 170 publications in physical organic, organometallic and stereochemistry. Chairman of the Symposium on Biological Homochirality (Serramazzoni, 1998).

Claudia ZUCCHI, PhD, Born 09.09.1964, San Prospero (Italy). Graduated at the University of Modena, actually external research associate of the Department of Chemistry of the same University. Author of 32 publications in organometallic and stereochemistry. Secretary of the Symposium on Biological Homochirality (Seffamazzoni, 1998).

Luciano CAGLIOTI, PhD, Dr.h.c. Born 13.11.1933 in Roma (Italy). Graduated at the University of Rome. Professor of Organic Chemistry at the University of Camerino (1964), Bologna (1968), Roma (1972). Author of cca 120 publications on the chemistry of natural substances. One of his preparative papers became "Citation Classic". Honorary member of the Hungarian Academy of Sciences (1995). Chairman of the Program Committee of the Symposium on Biological Homochirality (Serramazzoni, 1998).

Table of Contents

Advances in BioChirality
G. Pályi, C. Zucchi and L. Caglioti (Editors)

PREFACE

About two years ago, when the citizens and the Town Council of Serramazzoni learnt about the initiative of university professors belonging to various fields of natural sciences, to organize a Symposium on Biological Homochirality we suddenly realized that something important starts to happen. After the first meetings where these professors explained the main lines of this project we also got an insight into the main goals. The principal goal was not less than unifying forces from different fields to launch a "brain storming" towards one of the most delicate and at the same time decisive problems of the origin of life and of the function of living organisms.

Interdisciplinarity was the feature that impressed us the most. We are living in a World of specialists, with all of its advantages and drawbacks however we share the opinion of the organizers that really fundamental questions can be answered only on a broad intellectual basis, revitalizing the spirit of the "golden times" of the great scientific discoveries of the past century.

Another interesting feature of the programme expected a unified discussion of all present day chirality phenomena together with a look, as far as possible, in past ages, until not much later than the birth of the Earth, or even earlier!

This "horizontal" and "vertical" view, fits very well into the intellectual climate of Serramazzoni.

This small township is situated in 850 m altitude at the Northern side of the Appenines and its citizens are very proud of their direct descendence from the Ligurians (a pre-Roman nation, which can be traced back to the iron-age). Living Ligurian elements are also enriching our dialect. On the other side we are living in and actively participating in the developments of one of the technologically most avant-garde areas of Italy which include the high-tech automobile industry like nearby Ferrari, computer driven pig farms where two persons are caring for 5000 animals, or the highest level of automation in the World in the more than 300 ceramic factories in the Province of Modena.

The living link to our traditions, deep into the ages, is also realised in the production of such traditional wares like parmesan cheese or balsamic vinegar — obtained by methods practically unchanged in the last 2–3 millennia, and participating in development of technologies for the 21st Century this is our "vertical" and "horizontal" perspective. This atmosphere allowed our small township to find immediate contact with the philosophy of the organizers and then, later, with the scientists who honoured us by coming from three continents to participate at the Symposium on Biological Homochirality.

Particular thanks are due to the organizers, the sponsors and all of those persons who enthusiastically participated, sometimes ad-hoc in various aspects of the "management" of the Symposium. The wonderful activity of co-workers of the Associazione "Pro Loco" and of the staff preparing and running the Symposium site as well as of the students of the Course of Chemistry of the University of Modena and Reggio Emilia should be mentioned with particular accent. Without the scientific, technical and moral support of the University of Modena, its Faculty of Natural Sciences and its Department of Chemistry the Symposium could not even been initiated, not speaking of the successful results included in this Volume. Thanks are due to Elsevier for adopting this initiative.

Claudio Bartolacelli
Mayor of Serramazzoni
Serramazzoni, September 1998

Advances in BioChirality
G. Pályi, C. Zucchi and L. Caglioti (Editors)

CHAPTER 2

Dimensions of Biological Homochirality

Gyula Pályi,[1] Claudia Zucchi[1] and Luciano Caglioti[2]

[1] *Dipartimento di Chimica, Università di Modena, Via Campi 183, 41100 Modena, Italy;* [2] *Dipartimento di Studi di Chimica e Tecnologia delle Sostanze Biologicamente Attive, Università "La Sapienza", P.le A. Moro 5, 00185 Roma, Italy*

Introduction

The lack of a final answer (or answers ?) to the questions connected with the high degree of enantioselectivity in biological processes and the great variety of scientific disciplines involved in these problems prompted the organisers of the present Symposium to gather prominent representatives of research areas connected with the so-called "biological homochirality". In the following part of this review the main features of the viewpoints considered by the Organising Committee will be described.

The phenomenon which is often called "biological homochirality" manifests itself in high levels of preference for one of the enantiomers from possible two (for each centre of dissymmetry) in biological systems. This is a common feature of all living organisms (including viruses). Since the formation energies (enthalpy, Gibbs free energy) of enantiomers are strictly equal (within limits of measurement)*, this important particularity of life appears to be in contradiction with the laws of classical thermodynamics. Even if the modern thermodynamical description of open systems would allow mechanisms supposedly leading to the origin and maintenance of life, which includes homochirality as one of its most characteristic and inherent features, these problems represent still a high-level research challenge. At present excellent, highly intellectual theoretical solutions of the problems connected with the so-called biological homochirality are available [1]. However, these appear to be not yet the final solution, some are contradictory to each other and all are characterised by a certain scarcity of experimental verification. These theories and observations are products of very different fields of natural sciences.

* The calculated energy difference due to the asymmetry of neutral weak currents (see later) is very small, could not be directly measured yet.

Theory

There are several definitions of chirality, emphasising the absence of certain transformations [2] in the shape and/or position of geometric objects, point groups and functions. Additional theoretical problems emerge when proper mathematical formalism is searched for the description of these relationships [3].

Another theoretical approach to biological chirality puts the "starting point" of the questions to be asked (and answered) much earlier, about its origin. These theories are more of cosmological [4], astronomical [5], informatical [6] or even philosophical [7] nature, putting the solution of the problem of the origin of life itself [8] as the main goal of efforts.

A synthesis of the results of these fields would be highly desirable.

Extent, molecular manifestation

A convincing body of evidence indicates that a large majority of chiral molecules in living organisms is present in only one enantiomeric form.

The most clear-cut examples of this "rule" are the essential amino acids in proteins and natural peptides as well as the carbohydrate constituent of DNA and RNA [9].

The stereochemistry of other carbohydrates, steroids, several alkaloids and simpler terpene derivatives shows a high degree of homology but it is sometimes difficult to identify (without the exact knowledge of the biosynthetic routes leading to their formation) **which** atoms of these skeletons are to be correlated.

Presently we do not know exceptions to the homochirality rule in DNA or RNA but there is a rapidly increasing number of publications on the presence of D-amino acids in living organisms [10]. It is an exciting question to be answered, whether the presence of D-amino acids in biological systems is:

- a "record of the past" from a "racemic world";
- a product of natural defence mechanisms [11] or
- only a "typographical misprint"

or even a mixture of all of these?

The enantioselectivity [12] and sensitivity [12(d), 13] of analytical methods for detection and quantitative determination of chiral molecules of biological origin are among the key issues of this field.

Macroscopic manifestation

Several examples are known where living organism show chiral features of their body: parts of their body, disposal of their bodies (or parts of these) and their movements [14].

Well known examples include:

- human hands ("racemate") and their ability of operation ("diastereomeric"), the off-axial position of the heart of vertebrates;
- sense of winding (along an axis) of Helicobacter and of several plants as hop and beans (these latter show opposite "enantiomeric" helicity) [15];
- helicity of "spirogonites" of charophites [16];
- helicity of snails and mollusc shells [17] (generally showing very high but not quantitative "enantioselectivity");
- helical motion of some simple organisms (as Phytoflagellata or Euglena), but also of spermatozoa [18].

Not too much is known about the molecular background of these phenomena. Studies on genes causing the (very early!) left-right asymmetry in vertebrate embriogenesis [14] and identifications of some proteins steering biocrystallisation of some achiral materials as $CaCO_3$, $Ca_3(PO_4)_2$ or SiO_2 [19] represent an important starting runway but generate more open questions than solutions. Beyond curiosity for its own, answers to these open questions are also of very practical importance ranging from early detection of "genetic misprints" [20] to biomaterials industry [21].

Palaeochirality [22]

Theories and interpretations of model experiments about the origins of life are taking as one of the most fundamental "starting points" that life chemistry was enantioselective, consequently (chiral) biomolecules were enantiopure since very early times or even already from the early "prebiotic" period [23]. Arguments for this hypothesis are mostly based on the high level of enantioselectivity of **present-day** life and developmental or evolutionary interpretation of the history of life on the Earth (or elsewhere?). Even if the arguments justifying the "projection" of present-day biochemistry to Archaean or "prebiotic" conditions appear to be fairly well-founded **de facto** material evidences for terrestrial mechamism of enantioselectivity in the prebiotic period are lacking [24], while Archaean or more recent evidences are rare. There are two classes of evidences: (a) molecular and (b) macroscopic.

The molecular evidences for early appearance of biological chirality represent a particular class of the so-called "biomarkers" [25]. Unfortunately the biomarker-research often does not include the study of the enantiopurity even for compound classes, which are typically enantiopure in present-day organisms. Even with this sporadic character of the data, a fairly long record is available for enantiopure molecules [26,27] of biological origin [28]. According to the best of our knowledge the earliest enantiopure molecules (steroid derivatives) are dated to 520 Myr (B.P.) [29]. More sensitive materials, as proteins undergo much faster racemisation, generally do not survive with detectable enantiopurity 100–200 Kyr (B.P.) [30]. Even if local physical (temperature) and chemical (humidity, pH) factors influence the racemisation rate of fossil proteinaceous materials, where this rate depends also on the degree of depolymerisation suffered in fossilisation processes and on the chemical structure of the individual amino acids, the determination of the racemisation degree of fossil amino acids became an important method of sample age determination [31]. Obviously, this method, called amino[acid]chronology, suffers several sources of uncertainties, but it provides sometimes very useful results, if is complemented by other chronological studies, especially for comparison of samples of similar physical and chemical "history" [32].

The best conserved morphological and chemical state of all fossils are those included into amber. Attempts of "re-vitalisation" of fossil DNA from several Myr old amber inclusions, made newspaper headlines [33], and even more: these studies inspired the very successful "Jurassic Park". However, other groups raised later serious doubts about the reproducibility of these experiments [34]. The discussion appears to be not yet closed. Successful multiplication of more recent DNA samples may be, however, perhaps realised. It is an important recent result from the amber research, that the most ancient chiral protein (130 Myr B.P.) could be detected in an amber-derived sample [35].

It can reasonably be supposed, that macroscopic (morphological) chirality should be caused by biomolecular mechanisms including reactants of (partial or quantitative) enantiopurity [36]. This is the motive, why inorganic fossils of chiral morphology may be carriers of highly important indirect information about existence of molecular enantioselectivity in early stages of development of life on the Earth.

The first organisms which left chiral records (inorganic skeletons, or mineralised fossils) were perhaps *Ammonoidea*, a genus which became extinct near the end of the Cretaceous [37]. However these fossils are carriers of sporadic information.

Much more systematic source of information can be obtained from the morphology of the plants *Chara* (*Characea*) [16] and in (the animal world) of molluscs and snails [17].

Beyond the simple facts that these organisms are the first well-documented cases of macroscopic enantioselectivity, there are some additional challenging problems emerging in this field. One of the most spectacular of these is the "sudden" (~2–3 Myr) change of the sense of helicity of the spirogonites of all *Chara* species during the late Devonian "crisis" [38]. Since the real nature of this important crisis (changing several features of the living world) is not yet cleared up satisfactorily [41], there is a good chance that this chirality change could contribute to find a solution.

Theories of the Origin of Biomolecular Homochirality

A considerable number of theories about the origin of biomolecular enantioselectivity were published, most of them characterised by high-level intellectual deductions. Since it seems improbable that this problem has more than one solution, the fact that numerous equally valuable theories were presented, for us means, that no final answer had been achieved yet. However, several of these theories represent an important scientific contribution, and thus merits to be considered seriously. Here we can provide only some selected examples.

On the basis of traditional chemical experience, it is perhaps the most self-evident to look for mechanism in terms of modern theories of bifurcation kinetics and catalysis. In fact, important theoretical contributions were published on autocatalytic amplification of a very small excess of chirality [39]. More recently even the experimental observation of an enantioselective autocatalysis was reported [40]. This latter result can be regarded as one of the most important in the field.

An other "school" of scientists take into consideration a relatively new discovery in nuclear physics that the asymmetry of the weak neutral nuclear currents results in a small excess of energy in favour of one of the two enantiomers of chiral molecules [41]. Theoretical calculations indicate a preference in order of 10^{-17} for the naturally occurring L-amino acids and D-carbohydrates [41(d)], most recently it has been shown that this preference can be about 3 orders of magnitude higher [41(e)]. One counts that these small excesses of the preferential isomers could have been enriched by autocatalytic amplification mechanisms [39,40]. A related phenomenon is the polarised spontaneous β-radiation due to the asymmetry of the weak nuclear interaction. This might result in enantioselective radiolysis of racemates as it has been proved also experimentally [42].

Considerable attention is devoted to possibilities of extraterrestrial origin of biological homochirality [43] or even of life itself [43(c)]. Beyond the obvious philosophical importance of the "exportation" of our terrestrial problem into the Universe, these research efforts, intimately connected with several other fields of space research (e.g. the polarised synchrotron radiation from pulsars [44]) represent an important sector of the cosmological research projects. An interesting initiative [45] suggests that beyond systematic signal emission and research to and from space, the search of homochirality as an inherent signature of life (SETH) should be considered.

Practical aspects

The impact of research on biological chirality towards theoretical biology and biochemistry is (modestly saying) fundamental. Beyond these aspects in recent years several very practical features emerged giving rise to a new industrial field, so-called chirotechnology [46]. We should confine ourselves here only to compile a (non-complete) list of the main fields.

Pharmaceutical industry expects more efficient drugs as pure enantiomers and/or avoiding or at least minimalising unwanted side-effects [47].

Organic intermediate industry makes efforts to achieve better biodegradability through enantiopure products [48].

Polymer industry offers or hopes either more favourable technical parameters (e.g. higher degree of crystallinity) or new advantages (e.g. non-linear behaviour) by making polymers containing enantiopurity in the main chain [49].

Flavours and **fragrances** industry is proceeding towards developing new products on the basis of chiral interaction between the product molecules and the olfactory nerve sensors [50].

Materials industry is making efforts to obtain new, especially NLO materials from enantiopure starting materials or by enantioselective syntheses [51].

Obviously all these ambitions, and already introduced technologies, need chiral feedstocks. The need of preparation suitable intermediates (or starting materials) and the often high costs of additives, used for generating chirality, caused explosive development in **enantio-** and **diastereoselective catalysis** [52], as well as induced much interest in searching so-called chiral synthetic building blocks (synthons) [53]. Another secondary consequence is the rapid development of enantioselective **analytical**, especially **chromatographic** methods [54].

References

[1] Reviews: (a) S.F. Mason, Nature, 311 (1984) 19; (b) W.A. Bonner, Top. Stereochem., 18 (1988) 1; (c) W.A. Bonner, Chem. Ind. (1992) 640; (d) L. Keszthelyi, Quart. Rev. Biophys., 28 (1995) 473; (e) J. Jonas, Chem. Papers, 51 (1997) 167; (f) Scientific Am. Oct. 1994: Issue "Life in the Universe" Historical background; (g) V. Prelog, Science, 193 (1976) 17; (h) A.M. Amorim da Costa, Quimica (Lisbon), 60 (1996) 33.

[2] (a) P.G. Mezey, (Ed.), New Developments in Molecular Chirality, Kluwer, Dordrecht, 1991; (b) J.A. Goant, M.A. Gallard, B.T. Pickup, J. Comput. Chem., 17 (1996) 1653; (c) P.G. Mezey, Shape and Chemistry: An Introduction to Molecular Shape and Topology, VCH, New York, 1993; (d) C. Reidys, P.F. Stadler, Comput. Chem., 20 (1996) 85; (e) L.D. Barrow, Chem. Eur. J., 2 (1996) 743; (f) J. Jonas, New J. Chem., 21 (1997) 1251; (g) G. Moreau, J. Chem. Inf. Comput. Sci., 37 (1997) 929; (h) P.G. Mezey, Pharmaceutical News, 4 (1997) 29.

[3] (a) R.B. King, J. Math. Chem., 2 (1988) 89. (b) R.B. King, J. Math. Chem., 7 (1991) 69. (c) C. Reidys, P.F. Stadler, Comput. Chem., 20 (1996) 85; (d) V. Gontar, Chaos, Solitons, Fractals, 8 (1997) 517; (e) W. Qiu, Theochem., 397 (1997) 33; 401 151; (f) D. Avnir, D. Katzenelson, S. Keinan, M. Pinsky, Y. Pinto, Y. Salomon, H.Z. Hel-Or, Concepts Chem., (1997) 283.

[4] (a) N.-P. Chang, Looking 21st Century: 1st Joint Meet. Ch. Phys. Soc., Proc. Int. Conf. Frontiers of Physics 1995 (Publ. 1997) 784; (b) E.R. Harrison, Quart. J. Astr. Soc., 36 (1995) 193; (c) D.B. Cline, Comments Nucl. Part. Phys., 22 (1997) 131.

[5] J. Bailey, A. Chrisostomon, J.H. Hough, T.M. Gledhill, A. McCall, S. Clark, F. Ménard, M. Tamura, Science, 281 (1998) 672.

[6] (a) G.H.A. Cole, In: Entropy and Entropy Generation, J.S. Shiner (Ed.), Kluwer, Dordrecht, 1996, p. 129; (b), G. Arrhenius, B. Sales, S. Mojzsis, T.J. Lee, Theor. Biol., 187 (1997) 503; (c) M. Eigen, Proc. 5th Internat. Conf. Bioastronomy, 1996, C.B. Cosmovici, S. Bowyer, D. Werthimer (Eds.), Compositori, Bologna, 1997, p. 443; (d) M. Di Giulio, J. Theor. Biol., 187 (1997) 573; 191 (1998) 191; (e) Y. Irone, T. Wada, Adv. Supramol. Chem., 4 (1997) 55.

[7] (a) I. Prigogine, From Being to Becoming, Freeman, New York, 1980; (b) I. Prigogine, Lecture Univ. Siena, upon receiving Dr.n.c. in Biology, 10. Nov. 1990;. (c) M. Gell-Mann, The Quark and the Jaguar, Little, Brown & Co., London, 1994; (d) A. Garcia-Bellido, Proc. Natl. Acad. Sci. USA, 93 (1996) 14229; (e) K. Matsuno, Biosystems, 42 (1997) 129.

[8] (a) A. Salam, J. Mol. Evol., 33 (1991) 105; (b) K. Harada, Kagaku Kogyo (Tokyo), 45 (1992) 1253; (c) G. Gilat, Concepts Chem., (1997) 325; (d) A.J. MacDermott, Proc. SPIE – Int. Soc. Opt. Eug., 3111 (1997) 272; (e) S. Miki, Kagaku (Kyoto), 52(8) (1997) 70; (f) J.S. Siegel, Chirality, 10 (1998) 24.

[9] (a) D. Voet, J.G. Voet, Biochemistry (with 1991–1992 Supplements to Biochemistry), J. Wiley, New York, 1990; (b) W.H. Elliot, D.C. Elliot, Biochemistry and Molecular Biology, Oxford Univ. Press, Oxford, 1997.

[10] (a) N. Fujii, S. Muraoka, H. Kaoru, Viva Origino, 17 (1989) 119; (b) K. Harada, Viva Origino, 18 (1991) 97; (c) Y. Nagata, Viva Origino, 18 (1991) 131; (d) Y. Nagata, K. Kubota, Amino Acids, 4 (1993) 121; (e) Y. Nagata, R. Shoji, S. Yonezava, S. Oda, Amino Acids, 12 (1997) 95; (f) Y. Nagata, T. Fujiwara, K. Kawaguchi-Nagata, Y. Fukumori, T. Yamanaka, Biochim. Biophys. Acta, 1379 (1998) 76; (g) I. Gandolfi, G. Palla, R. Marchelli, A. Dossena, S. Puelli, C. Salvadori, J. Food Sci., 59 (1994) 152; (h) A. Albertini, T. Mentasi, V.M. Moretti, F. Bellagamba, U. Luzzana, F. Valfre, Milchwissenschaft, 51 (1996) 127; (i) K.H. Ekborg-Ott, D.W. Armstrong, Chirality, 8 (1996) 49.

[11] (a) G. Kreil, Annu. Rev. Biochem., 66 (1997) 337; (b) A. Huberman, Rev. Latinoam. Quim., 25 (1998) 103.

[12] (a) L. Labadini, R. Marchelli, A. Dossena, G. Palla, G. Mori, C. Bocchi, M. Pilone, S. Butto, Proc. 8th Eur. Conf. Food Chem., 1 (1995) 127; (b) A. Berthold, Y. Liu, C. Bagwill, D.W. Armstrong, J. Chromatogtr. A, 731 (1996) 123; (c) D. Fekkes, J. Chromatogr. B, 682 (1996) 3; (d) K. Imai, M. Kato, Y. Huang, H. Ichikara, T. Fukushima, T. Santa, H. Homma, Yukugaky Zasshi, 117 (1997) 637.

[13] (a) S.H. Lee, Y.H. Chang, K.P. Lee, Anal. Sci. Technol., 9 (1996) 52; (b) T. Pronce, B. Tilquin, J. Pharm. Biomed. Anal., 14 (1996) 1175; (c) H. Wan, A. Engstroem, G. Blomberg, J. Chromatogr. A, 731 (1996) 283; (d) M. Wada, N. Kuroda, S. Akiyama, K. Nakashima, Anal. Sci., 13 (1997) 945; (e) T. Fukusea, Chromatography, 18 (1997) 1.

[14] (a) N. Brown, L. Wolpert, Development, 109 (1990) 1; (b) W. Wood, Nature, 349 (1991) 536;. (c) T. Yokoyama, N.G. Copeland, N.G. Jenkins, C.A. Montgomery, F.F. Elder, P.A. Overbeek, Science, 260 (1993) 679 (d) M. Levin, R.L. Johnsom, C.D. Stern, M. Kuehn, C. Tabin, Cell, 82 (1995) 803; (e) M. Fujinaga, Int. J. Dev. Biol., 41 (1996) 153; (f) M. Levin, BioEssays, 19 (1997) 287; (g) W.B. Wood, Ann. Rev. Cell. Dev. Biol., 13 (1997) 53; (h) E.J. Robertson, Science, 275 (1997) 1280; (i) G. Vallortigara, A. Bisazza, Le Science, 342 (1997) 54; (j) M. Levin, M. Mercola, Genes & Dev., 12 (1998) 763; (k) A.K. Ryan, B. Blumberg, C.R. Esteban, S.Y. Tamura, K, Tamura, T. Tsukui, J. de la Pena, W. Sabbagh, J. Greenwald, S. Choe, D.P. Norris, E.J. Robertson, R.M. Evans, M.G. Rosenfeld, J.C.I. Belmonte, Nature, 394 (1998) 545.

[15] (a) A. Frey-Wyssling, Biol. Zeit., 5 (1975) 147; (b) M.F. Magdelin, D.K. Toomer, J. Ryan, J. Gen. Microbiol., 106 (1978) 73; (c) B.J. Marshall, J.R. Warren, Lancet, 1 (1983) 1311; (d) H. Quader, W. Herth, U. Ryser, E. Schnepf, Protoplasma, 137 (1987) 56; (e) T. Kamada, M. Tsuru, Mycol. Res., 97 (1993) 884; (f) M.J. Blaser, Trends Microbiol., 1 (1993) 255; (g) T. Kamada, The Mycota, 1 (1994) 367; (h) H.C. Crenshaw,