also prepared and treated the same way. This set acted as a control. Samples of one set were transferred to plastic petri dishes (8.5-cm diameter) on ice and photolyzed for 3 min with a ultraviolet lamp (115 volts, 60 Hz, 0.16 amp, Ultraviolet Products, Inc. San Gabriel, CA) at a distance of 15 cm (wavelength 265 nm). Controls were not exposed to UV light. After UV-light exposure, samples of both the sets were centrifuged at 50,000g for 10 min after adding 20 mL of buffer. The wash-dissociation cycles were repeated seven times, and binding of ligand to the receptor was determined as described above.

Acknowledgment. We acknowledge support of this work by the National Heart, Lung, and Blood Institute through research Grant HL-34052 to W. L. Nelson and by the Veterans Administration through a Merit Review grant to J. C. Giacomini.

Design and Synthesis of 14α-Methyl-15-aza-D-homosterols as Novel Antimycotics

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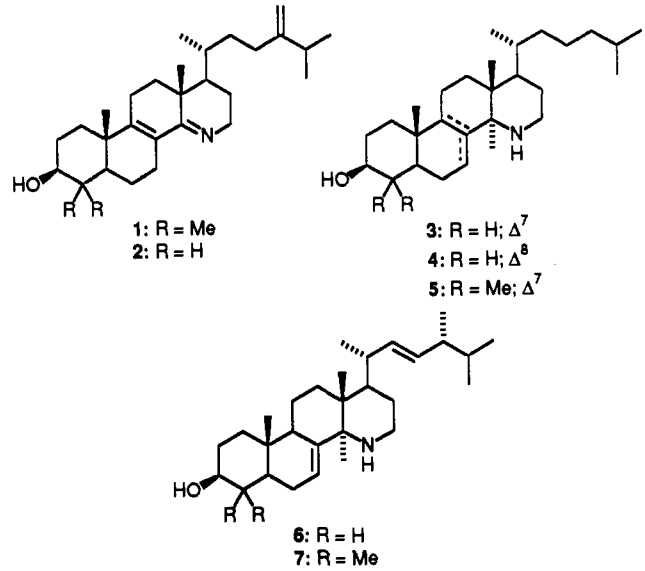
A novel series of 14α-methyl-15-aza-*D*-homosterols **3–7** has been synthesized. These compounds display significant antimycotic activity in vitro (MIC = 0.8–3.1 μg/mL) that compares quite favorably to the activity observed for fluconazole (MIC = 0.8 μg/mL). Azasterols **3** and **4** were active in vivo as reflected in the increased survival time of *Candida albicans* infected mice. The antimycotic activity of **3–7** is hypothesized to be a consequence of the inhibition of fungal 14,15-sterol reductase.

Over the past three decades an extensive amount of research has been devoted to the discovery of agents useful for the treatment of fungal infections in man.[1a] Particularly severe are the ever-increasing incidences of systemic mycoses, caused by various species of *Candida*, which occur in immuno-compromised and AIDS patients.[1bc] There is a great need for selective, orally active antimycotic agents that have a high therapeutic index.

Antimycotics either in clinical use or undergoing extensive clinical evaluation can be broadly grouped into two distinct classes: (1) those agents that disrupt membrane function (e.g. the ionophore antibiotics)[2] and (2) those agents that interrupt essential fungal enzyme processes and consequently are specific in their mode of action (e.g. the azoles).[1,2] The design and synthesis of fungal enzyme inhibitors presents itself as an appealing and tractable approach to the discovery of novel antimycotics. Such an approach is being explored in a number of laboratories as evidenced by the continued description of new inhibitors for a variety of fungal enzymes. Enzymes recently targeted for inhibition include: 14α-demethylase,[1,2] chitin synthetase,[3a] squalene epoxidase,[3b-d] squalene cyclase,[3e] and *S*-adenosylmethionine-Δ[24]-methyl transferase (24-SMT).[3f]

We have been interested in the enzyme 14,15-sterol reductase as a potential therapeutic target.[4] This enzyme, a salient member of the multienzyme cascade that is responsible for ergosterol biosynthesis in fungi and yeasts,[5a,b] has received little attention.[5c,d] Potent, naturally occurring inhibitors of the enzyme are known where A25822 factors A (**1**) and B (**2**) are representatives of this novel structural class of antimycotics.[6] The inhibition constant (K_i) for **2** against the enzyme is 2.0 nM, and both **1** and **2** exhibit impressive in vitro and in vivo antimycotic activity.[6b,d]

14,15-Sterol reductase is an NADPH-dependent enzyme that catalyzes the formal trans addition of hydrogen across the 14,15-π bond in 8,14-sterol dienes.[5c] Presumably, allylic carbocation (i) (Figure 1), a high-energy intermediate, is generated in the enzyme active site and is tightly bound to enzyme during catalysis. We have postulated that the high affinity of **1** and **2** for the enzyme is due to the structural similarity of protonated azasterols ii ↔ iii with intermediate i which is tightly bound to enzyme during



catalysis.[4] We reasoned that other sterols possessing a 15-aza-*D*-homo steroid nucleus may also be enzymatically

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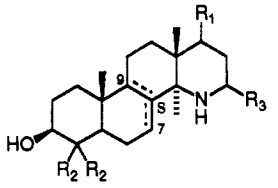
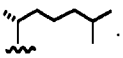
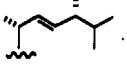
Table I. Structure, Physical Properties, and Anti-Candidial Activity of 14α-Methylazasterols[a]



compd	R_1	R_2	R_3	Δ	recrystn solvent	mp, °C	zone, size, mm[b] defined medium	zone, size, mm[b] complex medium	MIC[c]	MFC[c]	in vivo[b,d]
3	chol[e]	H	H	7	EtOAc/hexane	212–214	26	26	1.6	6.3	6/10
4	chol	H	H	8	EtOAc/hexane	206–207	22	18	3.1	6.3	4/10
5	chol	Me	H	7	EtOAc/hexane	173–174	26	22	1.6	3.1	
6	ergo[f]	H	H	7		amorphous	26	24	0.8	6.3	
7	ergo	Me	H	7	Et_2O/hexane	164–165	24	22	1.6	6.3	
23	chol	H	=O	7	Et_2O/hexame	221–223			>100		
fluconazole									0.8		
amphotericin									0.16		10/10

[a] All compounds exhibited satisfactory elemental analysis (C, H, N). [b] For *C. albicans* **3153A**. [c] Micrograms/millilter. [d] Number of survivors/total infected at day 14 after infection. Control animals expired within 7 days. [e] chol =  [f] ergo = 

protonated, resulting in the generation of analogous high-energy intermediate mimics (iv). Thus, as part of a program directed toward the design of steroid-based ergosterol biosynthesis inhibitors, a novel series of 14α-methyl-15-aza-*D*-homosterols **3–7** was prepared.

Chemistry

Oximination of the hindered C15-carbonyl present in sterols **8–12**[7] occurred using a large excess of NH_2OH·HCl under forcing conditions (120 °C; 3 days).[8a] Subsequent acidic workup afforded crystalline hydroxy oximes **13–17** (Scheme I). In the case of **9** → **14** it was necessary to remove the benzoate protecting group at C3 with use of NaOMe/MeOH. Each of the oximes underwent Beckmann rearrangement with $(CF_3CO)_2O$ in CH_2Cl_2 to furnish the *D*-homolactams **18–22**. Purifying these intermediates using flash chromatography was required, as byproducts were formed during the ring expansion.[8b] The desired 14α-methylazasterols **3–7** were readily obtained upon reduction of **18–22**, respectively, with LAH in refluxing THF. Hydroxy lactam **23** was quantitatively obtained following brief exposure of **18** to K_2CO_3 in MeOH.

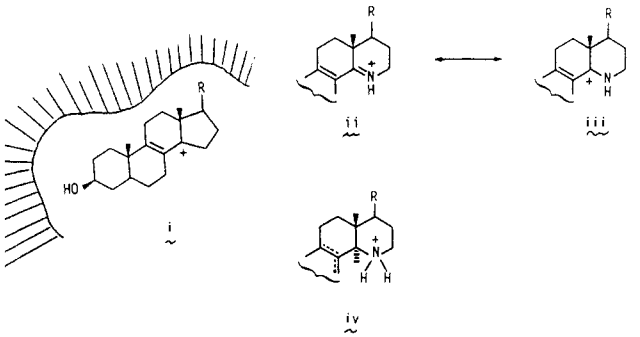


Figure 1. Hypothesized inhibition of 14,15-sterol reductase by azasterols **1–7**.

Results and Discussion

The in vitro and in vivo anti-Candidial activity of azasterols **3–7** and related lactam **23** are summarized in the Table I. In vitro fungistatic and fungicidal activity of these agents was determined by standard disk assays and by establishing minimum inhibitory and minimum fungicidal concentrations (MIC and MFC) with use of the *C. albicans 3153A* fungal strain.[9]

Analysis of the disk diffusion assays for **3–7** reveal sig-

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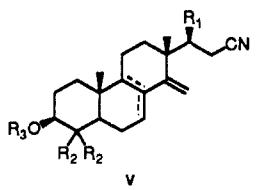
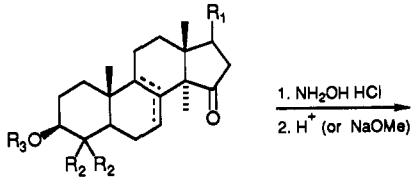
(8) (a) In each case, oximination of ketones **8–12** afforded a single oxime isomer (TLC, ^1H NMR). Inspection of molecular models suggests the geometry of the oxime to be anti with respect to C14. The syn isomer is destabilized realtive to the anti isomer due to a severe nonbonded interaction between the oxime oxygen atom and a proton at C7. (b) the Beckmann rearrangement also produced up to 40% of the secondary nitrile fragmentation products such as v. It is assumed that the 14α stereochemistry is retained during the Beckmann rearrangement based on the well-precedented stereospecificity of this rearrangement: Campbell, A.; Kenyon, J. *J. Chem. Soc.* **1946**, 25.



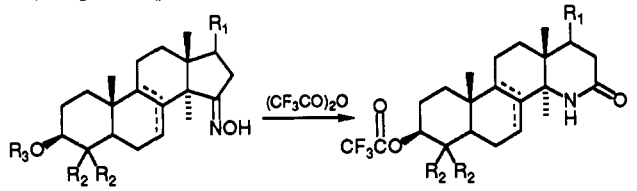
v

(9) (a) Kingsbury, W. D.; Boehm, J. C.; Mehta, R. J.; Grappel, S. F.; Gilvarg, C. *J. Med. Chem.* **1984**, *27*, 1447. (b) Shadomy, S.; Espinel-Ingroff, A.; Cartwright, R. Y. In *Manual of Clinical Microbiology*, 4th ed.; Lennette, E. H., Balows, A., Hausler, W. J., Jr., Shadomy, H. J., Eds.; American Society for Microbiology: Washington, D.C., 1985; Chapter 104. (c) Troke, P. F.; Andrews, R. J.; Brammer, K. W.; Marriott, M. S.; Richardson, K. *Antimicrob. Agents Chemother.* **1985**, *28*, 815.

Scheme I

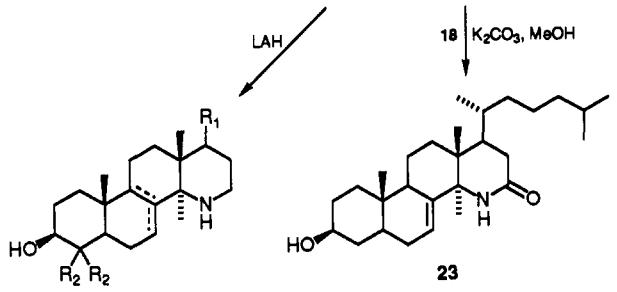


8: R_1 = chol, Δ^7; R_2 = H; R_3 = EE
9: R_1 = chol, Δ^8; R_2 = H; R_3 = Bz
10: R_1 = chol, Δ^7; R_2 = Me; R_3 = EE
11: R_1 = ergo, Δ^7; R_2 = H; R_3 = EE
12: R_1 = ergo, Δ^7; R_2 = Me; R_3 = EE



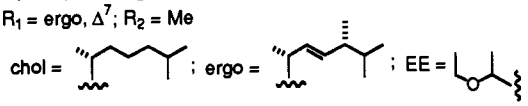
13: R_1 = chol, Δ^7; R_2 = H; R_3 = H
14: R_1 = chol, Δ^8; R_2 = H; R_3 = H
15: R_1 = chol, Δ^7; R_2 = Me; R_3 = H
16: R_1 = ergo, Δ^7; R_2 = H; R_3 = H
17: R_1 = ergo, Δ^7; R_2 = Me; R_3 = H

18: R_1 = chol, Δ^7; R_2 = H
19: R_1 = chol, Δ^8; R_2 = H
20: R_1 = chol, Δ^7; R_2 = Me
21: R_1 = ergo, Δ^7; R_2 = H
22: R_1 = ergo, Δ^7; R_2 = Me



23

3: R_1 = chol, Δ^7; R_2 = H
4: R_1 = chol, Δ^8; R_2 = H
5: R_1 = chol, Δ^7; R_2 = Me
6: R_1 = ergo, Δ^7; R_2 = H
7: R_1 = ergo, Δ^7; R_2 = Me



nificant fungistatic activity for these compounds against *C. albicans* grown on either defined or complex media. MIC's recorded for **3–7** range from 0.8 μg/mL[9c] (**6**) to 3.1 μg/mL (**4**). This activity compares quite favorably to the activity observed for fluconazole (MIC = 0.8 μg/mL)[9c] while these agents were less potent than amphotericin B. The azasterols display high fungicidal activity as evidenced by the MFC values (3–6 μg/mL) for **3–7**. The differences between the MIC and MFC values are minimal, indicating their therapeutic potential. The lactam **23** showed no antimycotic activity (Table I).

Two of the nitrogen-containing sterols, **3** and **4**, were selected for further evaluation in vivo on the basis of the therapeutic effect on the subacute murine model of candidiasis.[9] As shown in the Table I, in vivo efficacy of **3** and **4** was reflected in the increased survival time of *C. albicans* infected mice treated with a single 2 mg/kg dose (ip). After a 2-week time course, a 60% survival rate was measured for animals receiving **3**. Azasterol **3** was slightly more effective in prolonging survival than **4**. These results demonstrate that in vitro fungistatic activity of **3** and **4** corresponded well with in vivo activity.

The antimycotic activity of **3–7** is hypothesized to be a consequence of the inhibition of fungal ergosterol biosynthesis, specifically at the level of 14,15-reductase inhibition. Whether these novel 14α-methylazasterols inhibit this enzyme is still uncertain, as inhibition studies with purified enzyme have yet to be performed. There is no significant difference between the activities of **3–7**, and on the basis of the results available, a meaningful structure–activity relationship is precluded. It is clear, however, that a basic nitrogen is required for biological activity, since lactam **23** was found devoid of activity. This observation is consistent with our mechanistic contentions concerning the formation of iv, a high-energy mimic of i (Figure 1).

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. New compounds were routinely checked for their structure by IR (Perkin-Elmer 727; $CHCl_3$ thin film), ^1H NMR (Varian EM390; Me_4Si as internal standard), ^{13}C NMR (JEOL GX 270), and mass spectrometry (Finnigan Model 3625 equipped with chemical-ionization capability). Elemental analyses were performed in the SK&F Physical and Structural Chemistry Department (Philadelphia) and are within ±0.4% of the calculated values.

(3β,5α,14α)-3-Hydroxy-14-methylcholest-7-en-15-one 15-Oxime (13) and Analogues 14–17. A solution of **8**[7] (10 g, 20 mmol) in *tert*-amyl alcohol (120 mL) and pyridine (40 mL) containing 4-(dimethylamino)pyridine (12 g, 100 mmol) and NH_2OH·HCl (25 g, 360 mmol) was refluxed for 3 days. The reaction mixture was cooled and poured into 1 N aqueous H_2SO_4 (1 L)/ice, and the mixture was extracted with ether (3 × 200 mL). The ether extracts were combined and washed with 1 N aqueous H_2SO_4 (5 × 200 mL), saturated aqueous $NaHCO_3$ (2 × 200 mL), water (5 × 200 mL), and brine (200 mL) and dried ($MgSO_4$). Removal of the solvent in vacuo gave a solid residue, which was thrice recrystallized (ether/hexane) to provide 6.6 g (76%) of oxime **13**: mp 218–219 °C; MS *m/e* (relative intensity) 428 (M − 1, 42), 412 (100), 394 (7).

Compounds **9–12** underwent the analogous oximination reaction. In the case of **9** → **14**, the residue obtained upon removal of the solvent (following oximination) was dissolved in 3:1 methanol/toluene and exposed to excess NaOMe (10 equiv, 12 h, 25 °C). Standard workup and flash chromatography afforded **14** in good yield (72%).

(3β,5α,14α)-14-Methyl-3-[(trifluoroacetyl)oxy]-15-aza-*D*-homocholest-7-en-16-one (18) and Analogues 19–22. Trifluoroacetic anhdyride (0.65 mL, 4.6 mmol) was added to a cooled (0 °C) solution of oxime **13** (1 g, 2.3 mmol) in CH_2Cl_2 (3 mL). The reaction mixture was warmed to 25 °C and stirred for 15 min, and then the solvent and excess acid anhydride were removed in vacuo. Flash chromatography (50% EtOAc/hexane) of the residue gave 732 mg (60%) of lactam **18**. The Beckmann rearrangement of **14–17** was conducted similarly. For **18**: foam; MS *m/e* (relative intensity) 526 (M + 1, 98), 430 (4), 412 (100). Anal. $C_{30}H_{46}F_3NO_3$: C, H, N.

(3β,5α,14α)-3-Hydroxy-14-methyl-15-aza-*D*-homocholest-7-en-16-one (23). Lactam **18** (100 mg, 0.2 mmol) was dissolved in methanol (1 mL) containing powdered K_2CO_3 (105 mg), and the reaction mixture was stirred for 2 min at room temperature. Filtration and removal of solvent in vacuo gave 77 mg (90%) of **23** following recrystallization (ether/hexane): mp 251–253 °C; MS *m/e* (relative intensity) 430 (M + 1, 55), 412 (100), 394 (67). Anal. $C_{28}H_{47}NO_2$: C, H, N.

(3β,5α,14α)-14-Methyl-15-aza-*D*-homocholest-7-en-3-ol (3) and Analogues 4–7. A solution of **18** (1 g, 1.9 mmol) in THF (30 mL) containing LAH (144 mg, 3.8 mmol) was reluxed for 12 h. The reaction was cooled (0 °C) and excess LAH destroyed via the cautious addition of 3:1 THF/water. The reaction was diluted with THF (25 mL) and dried ($MgSO_4$). Filtration and removal of the solvent in vacuo gave 700 mg (70%) of analytically pure **3** after a subsequent recrystallization (EtOAc/hexane). The analogous reductions of **19–22** produced **4–7** (Table I). For **3**: mp 212–214 °C; MS *m/e* (relative intensity) 416 (M + 1, 48), 398 (100). Anal. $C_{28}H_{49}NO$: C, H, N.

Fungistatic/Fungicidal Activity. Each compound was tested for fungistatic/fungicidal activity against *Candida albicans* 3153A. Disk assays were determined with cultures grown on

defined (YCB/lysine broth) and complex (Sabouraud dextrose broth) media as previously described.[9a,b] MIC and MFC values were also determined as previously described.[9] Minimum fungicidal activity (MFC) was defined as the lowest concentration of drug from which subcultures were negative or which yielded fewer than three colonies.[9b]

Systemic Infections with *Candida albicans* in Mice.[9c] Typically, 6×10^6 yeast cells of *C. Albicans 3153A* were injected into a tail vein of mice (weight: 20–22 g) to generate a subacute systemic model. Untreated animals expired within 7 days. For the therapeutic test, 2 mg of **3** and 4 or 1 mg of amphotericin B was administered once intraperitoneally on day 2 (2 days after the challenge). Efficacy was evaluated by the survival of mice on day 14.

Registry No. **3**, 123290-45-9; **4**, 123290-46-0; **5**, 123307-60-8; **6**, 123290-47-1; **7**, 123290-48-2; **8**, 123290-49-3; **9**, 123290-50-6; **10**, 123290-51-7; **11**, 123290-52-8; **12**, 123290-53-9; **13**, 123290-54-0; **14**, 123290-55-1; **15**, 123356-30-9; **16**, 123290-56-2; **17**, 123290-57-3; **18**, 123290-58-4; **19**, 123290-59-5; **20**, 123290-60-8; **21**, 123307-97-1; **22**, 123290-61-9; **23**, 123290-62-0; 14,15-sterol reductase, 69403-07-2.

Supplementary Material Available: ^{1}H NMR, MS, and TLC data for compounds **13–22** and **3–7** (1 page). Ordering information is given on any current masthead page.

Additions and Corrections

1989, Volume 32

Whei-Mei Wu, Emil Pop, Efraim Shek, and Nicholas Bodor*: Brain-Specific Chemical Delivery Systems for β-Lactam Antibiotics. In Vitro and in Vivo Studies of Some Dihydropyridine and Dihydroisoquinoline Derivatives of Benzylpenicillin in Rats.

Pages 1785–1786. During the printing process, Figures 2 and 3 were reversed (the captions are correct). In both figure captions the last symbol should be changed from ▽ to ○.

Book Reviews

The Muscarinic Receptors. Edited by Joan Heller Brown. Humana Press, Clifton, NJ. 1989. xviii + 478 pp. 15 × 23 cm. ISBN 0-89603-156-X. $89.50.

This is the sixth book in *The Receptors* series. Research on muscarinic receptors has been particularly intense during the past decade. It is now accepted that a family of these receptors exist. Advances of the understanding in this very important class of receptors are presented in 12 chapters written by experts in the field. These chapters present a wealth of information on a wide variety of topics including the history and fundamental properties of the muscarinic cholinergic receptors, their binding and pharmacological properties, purification, subpopulations, cloning, structural determinants of muscarinic agonist activity, regulation of cyclic AMP and phospholipid metabolism, calcium mobilization, allosteric interactions, regulation of cyclic GMP, eicosanoid production, ion channels, and the number and function of this class of receptors. The book concludes with an excellent discussion of future research anticipated for the muscarinic receptors.

Each chapter is followed by an excellent up-to-date list of references. An author index is also included. *The Muscarinic Receptors*, in keeping with other volumes in the series, is essential reading for those concerned with this class of receptors. Medicinal chemists will find chapter 5, Structural Determinants of Muscarinic Agonist Activity, to be a comprehensive review of particular interest.

Staff

Organic Functional Group Preparations. Volume III. Second Edition. By Stanley R. Sandler and Wolf Karo. Academic Press, San Diego, CA. 1989. xiv + 512 pp. 16 × 23.5 cm. ISBN 0-12-618603. $99.00.

The purpose of this series is to provide organic chemists with an up-to-date and convenient source of useful preparative procedures. For this second edition the literature for 13 functional groups has been reviewed from 1971 to the present. It includes new information where pertinent, new and expanded tables of data, and additional preparations. References are derived from journal as well as United States and foreign patent literature.

Topics included in the present volume are acetals and ketals, anhydrides, monoalkyl sulfates, and sulfenic acids and sulfenic acid derivatives, isonitriles (isocyanides), amidines, imides, imidates, nitrones, hydroxylamines and substituted hydroxylamines, oximes, hydroxamic acids, and thiohydroxamic acids. Where possible, the preparative details for each functional group are divided according to their reaction type, i.e., condensation, elimination, oxidation, and reduction.

This volume of *Organic Chemistry, A Series of Monographs, Volume 12-III* is of general organic synthetic importance. It will be a valuable addition to the libraries of medicinal chemists with focus on any of the 13 functional group preparations that are reviewed.

Staff

Molecular Structure and Energetics. Volume 9. Mechanistic Principles of Enzyme Activity. Volume 10. Environmental Influences and Recognition in Enzyme Chemistry. Edited by Joel C. Liebman and Arthur Greenberg. VCH Publishers, Inc., New York, NY and VCH Verlagsgesellschaft mbH, Weinheim, Federal Republic of Germany. 1988. Vol. 9: xii + 404 pp. 16 × 24 cm. ISBN 0-89573-706-x. $89.00. Vol 10: xv + 349 pp. 16 × 24 cm. ISBN 0-89573-707-8. $89.00.

These two volumes continue the title series with a focus on the influence of structure and energetics upon activities and properties of selected enzyme systems. The first of these books is intended to deal primarily with mechanistic principles, including experimental methods that have been employed in their study, and the second with structural and environmental influences on enzyme catalysis, including emphasis on theoretical considerations. The segregation of the included topics into two classifications,