Cannabidiol and Phenytoin: A Structural Comparison

Ilana Tamir,* Raphael Mechoulam,

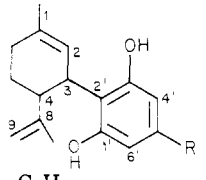
Department of Natural Products, School of Pharmacy

and Amatzya Y. Meyer

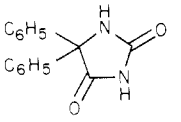
Department of Organic Chemistry, Hebrew University, Jerusalem, Israel. *Received May 8, 1979*

Conformational energy maps have been computed for the antiepileptic agents phenytoin and cannabidiol by the quantum-mechanical method of perturbative configuration interaction with localized orbitals (PCILO). The computation indicates that the spatial relationship between the two rings in the two drugs is similar and close to the respective structures in the crystal. This is supported by ^{1}H and ^{13}C NMR measurements. Hence, both compounds fulfill the stereochemical requirements suggested for anticonvulsant drug action.

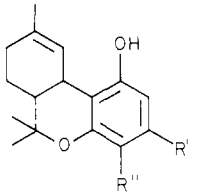
Cannabidiol (CBD, I) is a major component of most



I, R = C_5H_{11}
VI, R = $CH(CH_3)CH(CH_3)C_5H_{11}$



II



III, R' = C_5H_{11}; R'' = H
IV, R' = C_5H_{11}; R'' = COOH
V, R' = $CH(CH_3)CH(CH_3)C_5H_{11}$; R'' = H

cannabis preparations.[1] It causes none of the typical cannabis effects when administered to animals or man.[1,2] However, it is an anticonvulsant in several animal species.[3] Recently it was demonstrated that it is a potent antiepileptic agent in man.[2]

In a series of publications, Camerman and Camerman have presented crystallographic evidence that several chemically different drugs, such as phenytoin (diphenylhydantoin, II),[4] diazepam,[4] procyclidine,[5] trihexyphenidyl,[6] ethylphenacemide,[7] and and diphenylsilanediol,[8] which possess clinical or laboratory demonstrated activities against grand mal epilepsy, have closely related conformational features. They all contain two hydrophobic moieties which, in some crystal structures, have closely similar relative orientations and are thus maximally superimposable. Each compound has two electron-donor groups situated in similar orientations and positions with respect to each other and to the hydrophobic groups. These conformational features have been suggested as necessary for anticonvulsant activity of the phenytoin type.[4]

It seems of interest to compare the three-dimensional structures of cannabidiol and phenytoin in order to determine any possible relationship between them. The establishment of a conformational similarity between these two agents could eventually help in understanding the molecular basis of cannabidiol activity assuming that the same class of receptors is involved.

Cannabidiol has been examined by crystallography.[9,10] Its spatial conformation is indeed related to that of phenytoin. However, on reinvestigation of the common stereochemical features of some of the antiepileptic drugs previously studied by Camerman and Camerman, it was concluded that the previously postulated requirements concerning the number and orientation of the hydrophobic rings (in the crystalline state) appeared to be too specific and that further investigations using different techniques were desirable.[11] Furthermore, dissolution of a molecule might be attended by conformational changes, so that the reacting species is not necessarily identical in conformation with the isolated or crystalline form. It is therefore of consequence to establish not only the conformation of the isolated molecule or the molecule in a crystal but also that of the molecule in solution.

Here we compare the stereochemistry of cannabidiol and phenytoin, starting with a theoretical conformational analysis of the *free* molecules. An NMR analysis, which obviously refers to the *solvated* species, then follows. The two approaches complement and substantiate each other.

Cannabidiol and phenytoin were calculated by PCILO ("perturbative configurational interaction with localized orbitals"),[12] a rapid semiempirical all-valence-electron method that had been applied extensively to molecules of biological interest.[13]

(1) R. Mechoulam, Ed., "Marihuana Chemistry, Pharmacology, Metabolism and Clinical Effects", Academic Press, New York, 1973.
(2) R. Mechoulam and E. A. Carlini, *Naturwissenschaften*, **65**, 174 (1978).
(3) E. A. Carlini, J. R. Leite, M. Tannhauser, and A. C. Berardi, *J. Pharm. Pharmacol.*, **25**, 644 (1973); I. Izquierdo, O. A. Orsinger, and A. C. Berardi, *Psychopharmacology*, **28**, 95 (1973); S. A. Turkanis, W. Cely, D. M. Olsen, and R. Karler, *Res. Commun. Chem. Pathol. Pharmacol.*, **8**, 231 (1974); P. Consroe and A. Wolkin, *J. Pharmacol. Exp. Ther.*, **201**, 26 (1977).
(4) A. Camerman and N. Camerman, *Science*, **168**, 1457 (1970); A. Camerman and N. Camerman, *Acta Crystallogr., Sect. B*, **27**, 2205 (1971); A. Camerman and N. Camerman, *J. Am. Chem. Soc.*, **94**, 268 (1972).
(5) N. Camerman and A. Camerman, *Mol. Pharmacol.*, **7**, 406 (1971).
(6) N. Camerman and A. Camerman, *J. Am. Chem. Soc.*, **94**, 8553 (1972).
(7) A. Camerman and N. Camerman, *Proc. Natl. Acad. Sci., U. S.A.*, **74**, 1264 (1977).
(8) J. K. Fawcett, N. Camerman, and A. Camerman, *Can. J. Chem.*, **55**, 3631 (1977).
(9) P. G. Jones, L. Falvello, O. Kennard, G. M. Sheldrick, and R. Mechoulam, *Acta Crystallogr., Sect. B*, **33**, 3211 (1977).
(10) T. Ottersen, E. Rosenqvist, C. E. Turner, and F. S. El-Feraly, *Acta Chem. Scand., Ser. B*, **31**, 807 (1977).
(11) P. G. Jones and O. Kennard, *J. Pharm. Pharmacol.*, **30**, 815 (1978).
(12) B. Pullman, B. Maigret, and D. Perahia, *Theor. Chim. Acta*, **18**, 44 (1970).

0022-2623/80/1823-0220$01.00/0

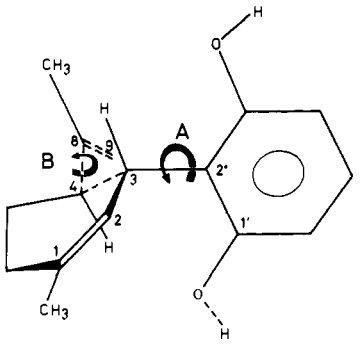


Figure 1. Cannabidiol: starting conformation and senses of internal rotation. Initial *A*, −72°; initial *B*, +117°.

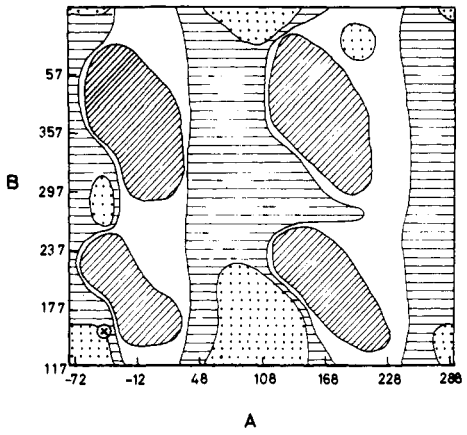


Figure 2. Cannabidiol: potential surface (kcal mol^{-1}, PCILO). The minimum energy conformation is indicated by a cross. Dotted: ranges less than 3.5 kcal mol^{-1} above minimum; lined: 3.5–10 kcal mol^{-1}; bare: 10–25 kcal mol^{-1}; densely lined: crests.

Calculations

Cannabidiol (I). In I, there are (apart from the pentyl side chain) two axes of internal rotation: phenylcyclohexenyl (axis *A*, Figure 1) and cyclohexenylisopropenyl (axis *B*). In the calculations, the phenyl group was held in place, and the cyclohexenyl was rotated around axis *A* with respect to the phenyl, in the sense marked in Figure 1. Concurrently, the isopropenyl group was rotated around axis *B* with respect to the cyclohexenyl, in the sense marked. Rotations about axes *A* and *B* are tantamount to changes in the dihedral angles $\omega(1'\text{–}2'\text{–}3\text{–}2)$ and $\omega(3\text{–}4\text{–}8\text{–}9)$, respectively. The hydroxyl groups on the aromatic nucleus were included in the calculation but, in order to allow for geometrical variability, were not taken in the C_{2v} local relationship. Because of computing limitations, the pentyl side chain was not included.

The starting conformation was that of "molecule 1" in the crystal[9] and is shown in Figure 1. The relevant dihedral angles are $\omega(1'\text{–}2'\text{–}3\text{–}2) = +54°$, $\omega(1'\text{–}2'\text{–}3\text{–}4) = -72°$, and $\omega(3\text{–}4\text{–}8\text{–}9) = +117°$, where a positive sign indicates a clockwise rotation from the eclipsed position.[14]

Rotations were performed by steps of 30°, about both axes *A* and *B*. A finer grid (10°) was taken in vicinity of the minimum. The energies we computed and report herein correspond, therefore, to "rigid rotations" and not to relaxed structure.[15] As such they are certainly over-

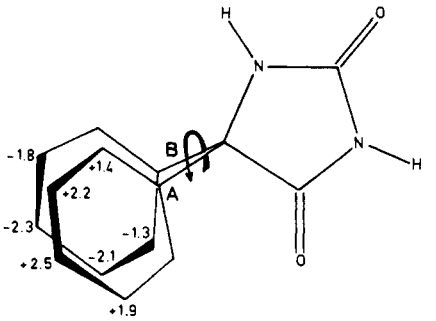


Figure 3. Phenytoin: minimum-energy conformation of phenytoin. The hydantoin ring is in the plane of the paper. Marked are the two axes of rotation, *A* and *B*, and the sense of rotation about the axes. Also given is the elevation (in Å units) of key carbon atoms in the phenyl rings.

estimated.[16,17] Still, we consider the results representative as concerns the regions of maxima and the trends in the vicinity of minima. The actual numbers computed, near and at maxima, are of limited significance.

The potential surface is shown in Figure 2, where the origin corresponds to the crystal structure. For convenience, four types of range have been distinguished: (a) computed energy up to 3.5 kcal mol^{-1} above the minimum; (b) energies from 3.5 to 10 kcal mol^{-1}; (c) structures of higher energy content (cutoff at 25 kcal mol^{-1}); (d) crests which may be considered as unsurmountable.

As Figure 2 shows, there are two rather wide zones of low energy. The lowest energy computed (denoted in Figure 2 by a cross, $A \approx -42$, $B \approx 157°$) differs from the energy computed for the crystal geometry ($A = -72$, $B = 117°$) by 3.5 kcal mol^{-1} (only about 3.5×10^{-3} percent of the total energy, which is of the order of −100 000 kcal mol^{-1}). For this conformation, the angle between the phenyl ring and a plane through atoms 1, 2, and 3 in the cyclohexenyl ring is 79°. The energy valley is flat, so that no particular consequence can be attributed to the deviation ($\Delta A \approx 30$, $\Delta B \approx 40°$) of the computed from the crystal geometry. The two minimum-energy zones are 180° apart, interconversion being tantamount to internal rotation about axis *A*.

From Figure 2, hindrances to internal rotation about *A* are expected to be detectable on the NMR time scale (see below). When the dihedral angle about axis *B* is close to the crystal value, the barriers for rotation about axis *A* are of the order of 20 kcal mol^{-1}. They are encountered when the isopropenyl substituent approaches one or the other of the phenylic hydroxyls, that is, at ca. 0° and 210° about axis *A*.

On the other hand, rotation about *B*, for *A* in the range from −72° to −100° (i.e., +260° in Figure 2), is comparatively free; the computed maxima in this range are within 7 kcal mol^{-1} of the global minimum. They are encountered at *B* rotations of 240° and 60°, when either the isopropenylic methylene or methyl approaches the aromatic ring.

It may also be gathered from Figure 2 that extensive conformational ranges are accessible to the free molecule; it cannot be considered as rigid. Yet, there are four domains (dense lines in the figure) which should be consid-

(13) B. Pullman, *Isr. J. Chem.*, 14, 69 (1975).
(14) W. Klyne and V. Prelog, *Experientia*, 16, 512 (1960).
(15) S. Mizushima, "Structure of Molecules and Internal Rotation", Academic Press, New York, 1954, p 56; N. L. Allinger, M. T. Tribble, M. A. Miller, and D. H. Wertz, *J. Am. Chem. Soc.*, **93**, 1637 (1971).
(16) J. P. Colpa, H. B. Schlegel, and S. Wolfe, *Can. J. Chem.*, **54**, 526 (1976).
(17) This is particularly noticeable in the vicinity of crests, where the close approach of two atoms produced computationally an unrealistically high nuclear repulsion, much of which is actually relievable by relaxation in other degrees of freedom (e.g., narrowing or widening of valence angles).

ered as inaccessible. In these, the cyclohexene and the isopropenyl are rotated such that either the methyl or the methylene virtually overlaps with either of the hydroxyls on the aromatic ring.

Phenytoin (II). The starting conformation of phenytoin was that of the crystal,[4] and we computed the energy changes due to rotation of the two phenyl rings about their axes, using steps of 10°. The most stable conformation is shown in Figure 3. It is quite close to the conformation in the crystal, with one phenyl ring (A) rotated by 20°, the other (B) rotated by 170° with respect to the crystal. The angle between the two phenyl rings is 67°, vs. 90° in the crystal. There is a second minimum at a rotational angle of 200° for the first phenyl (that is, 180° from the first minimum), with the other phenyl as before (170°). Because of a slight distortion of the phenyl rings in the crystal data, the second minimum is 0.84 kcal mol^{-1} above the first (out of a total energy of about −110 000 kcal mol^{-1}). The minima are separated by an unsurmountable barrier. The energy valleys in phenytoin are very steep: rotation by 20° of either phenyl from the minimum is accompanied by an energy increase of at least 10 kcal mol^{-1}. Hence, the calculations imply that the two minima are not interconvertible. This is corroborated by NMR.

Nuclear Magnetic Resonance of Cannabidiol and Phenytoin

Δ^1-Tetrahydrocannabinol (Δ^1-THC, III) has a rigid structure, with the three rings virtually coplanar.[18] As a consequence, the two aromatic protons (δ 6.00, 6.18 ppm) are nonidentical. In cannabidiol, however, only a single aromatic peak is observed, indicating a noncoplanar spatial arrangement. Hence, experimental evidence for the stable spatial configuration of cannabidiol was sought by comparing its ^{1}H NMR spectrum with that of Δ^1-THC. In chloroform solution, the chemical shifts of Δ^1-THC are: C_2-H, δ 6.35; C_3-H, δ 3.14 ppm.[18] This indicates a strong deshielding as compared to the corresponding protons in cyclohexene (5.59 and 1.59 ppm, respectively).[19] It can be rationalized as due to the presence of the aromatic ring and the various functional groups. Analogous deshielding effects have been observed in other molecules.[20] In cannabidiol, where rotation about the intercyclic bond is restricted, other values are observed: C_2-H is shifted upfield to δ 5.54 ppm, while C_3-H shifts downfield to 3.85 ppm. Apart from its sign, the change in chemical shift is very similar for the two protons, +0.81 for C_2-H and −0.71 ppm for C_3-H. These reversals can be attributed to the change in ring-current contribution to the shielding. They reflect the reversal in the relative geometric position of protons C_2-H and C_3-H (in cannabidiol) with regard to the plane of the aromatic ring.[18]

For a more quantitative estimate, the distances and dihedral angles were calculated for both protons in cannabidiol and Δ^1-THC. In Δ^1-THC, which is amorphous in the solid, the reported geometry[21] of the crystalline Δ^1-THC acid, *B* (6′-carboxy-Δ^1-tetrahydrocannabinol, IV), was used. In cannabidiol, we took the minimum energy conformation, as computed by PCILO for the single molecule (above), which corresponds to a −30° rotation about *A*, −40° about *B*, with respect to the crystal. The distances of the protons in question from the center of the aromatic ring are very similar in the two molecules: C_2-H is located at 3.37 in cannabidiol and 3.50 Å in Δ^1-THC, C_3-H being at 3.79 and 3.62 Å, respectively. A reversal in the dihedral angles of these atoms with respect to the benzene plane occurs in the two molecules. The proton attached to C_2 in Δ^1-THC has a dihedral angle of 5.8° with respect to the aromatic ring, as compared with 58.3° in cannabidiol, while the proton on C_3 changes from 74.4° in Δ^1-THC to 20.5° in cannabidiol. Thus, the transition of a proton from a nearly perpendicular to a nearly coplanar position is accompanied by a chemical-shift change of 0.7–0.8 ppm.

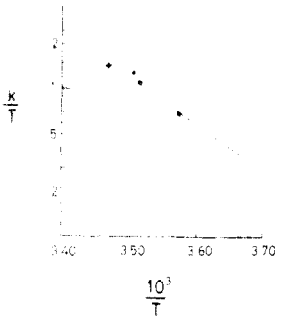


Figure 4. Cannabidiol: Eyring plot.

A theoretical estimate of the ring-current contribution to the shielding yielded an average value of 0.6 ppm. This calculation was performed by the Mallion procedure;[22] the ring current was taken as 0.1 as in benzene.[23] Thus, the measured chemical shifts support the conclusion, reached by PCILO calculations, that the stable conformation of the *single* molecule of cannabidiol is rotated with respect to Δ^1-THC, as well as (though to a much smaller extent) to cannabidiol in the *crystalline* state.

Another prediction of the theoretical calculation is that there are two minima (since cyclohexene is not substituted symmetrically) and that the barrier to internal rotation about the intercyclic axis is of the order of 20 kcal mol^{-1}. This could be tested through a dynamic NMR study of cannabidiol. At room temperature, both aromatic protons, $C_{4'}$- and $C_{6'}$-H, appear as a sharp singlet at δ 6.25 ppm, which splits upon cooling into two separate peaks of equal intensity. The separation between these peaks increases upon cooling, becoming constant at 0 °C. Assuming a process of exchange between two environments and utilizing the approximate method of Gutowsky and Holm,[24] we calculated the rate constants for this process at several temperatures.

The Eyring plot, Figure 4, is a straight line with a correlation coefficient of 0.97. The enthalpy and entropy of activation are $\Delta H^\ddagger$ = 11.5 kcal mol^{-1} and $\Delta S^\ddagger$ = 16.3 eu, and the free energy of activation for coalescence at +15 °C is $\Delta G^\ddagger$ = 6.8 kcal mol^{-1}. The $\Delta H^\ddagger$ value of 11 kcal mol^{-1} obtained from the NMR spectra is lower than the value derived theoretically. The latter obviously must be considered as an upper limit, since it was obtained under the assumptions of rigid rotation and nonsolvation. As such, the agreement between the NMR and the PCILO estimation is reasonable.

^{1}H and ^{13}C NMR measurements also support the theoretical structure for the free molecule of phenytoin. Proton

(18) Y. Gaoni and R. Mechoulam, *J. Am. Chem. Soc.*, 86, 1646 (1964).
(19) L. M. Jackman and S. Sternhell, "Applications of NMR Spectroscopy in Organic Chemistry", 2nd ed, Pergamon Press, Oxford, 1969, p 188.
(20) A. C. Huitric, W. G. Clarke, Jr., K. Leigh, and D. C. Staiff, *J. Org. Chem.*, 27, 715 (1962).
(21) F. Rosenqvist and T. Ottersen, *Acta Chem. Scand., Ser. B*, 29, 379 (1975).
(22) R. B. Mallion, *J. Chem. Soc. B*, 681 (1971).
(23) R. McWeeny, *Mol. Phys.*, 1, 311 (1958).
(24) H. S. Gutowsky and C. H. Holm, *J. Chem. Phys.*, 25, 1228 (1956).

resonance was measured for solutions in Me_2CO-d_6 and Me_2SO-d_6. Two similar peaks at a separation of 0.125 ppm were recorded for the aromatic protons at room temperature in Me_2CO-d_6. At the same temperature, in Me_2SO-d_6 a broad single peak ($\Delta\nu_{1/2}$ = 2 Hz) was observed for the phenyl protons. Elevation of temperature caused this singlet to split in two, with increasing separation. This was taken to indicate phenyl anisotropy, not the presence of two conformers. This conclusion is supported by the ^{13}C NMR spectra of phenytoin in Me_2SO-d_6. Various types of carbon could be distinguished, but only a single peak was observed at the aromatic range, at 110 ppm. This is in line with a single spatial conformation in phenytoin: the anisotropy of the phenyls (observed in the ^1H NMR) is due to a long-range contribution to the shielding by the two anisotropic carbonyl groups; this contribution is negligible for the ^{13}C chemical shift.[25] Thus, both the theoretical calculations for the isolated molecule and the NMR spectrum of solvated phenytoin indicate that the drug has a single conformation, with the two phenyls rotated significantly with respect to each other.

Conclusion

In summary, the theoretical computation of free cannabidiol and phenytoin indicates that in these compounds the angle between the two rings is close to, but rotated with respect to, the crystal. It is of interest that the changes in both molecules are in the same direction and of similar magnitude. NMR measurements of the solvated molecules support the theoretical prediction.

The data above indicate a close relationship between cannabidiol and phenytoin as regards the spatial relation of the two rings; hence, they are in line with the stereochemical requirements suggested for anticonvulsant drug action. This encompasses also the second requirement, namely, on the similar orientations and positions of the two electron-donating groups: in cannabidiol, the distance between the two groups is 4.77 Å;[9-11] in phenytoin, it is 4.56 Å.[4]

It is of some interest to point out that while the hashish-type activity of THC increases sharply when the pentyl side chain is replaced by 1,2-dimethylheptyl (for example, in compound V), no difference in anticonvulsant activity is observed when a similar change is made in the cannabidiol molecule (for example, in compound VI).[27] This absence of enhancement of anticonvulsant activity is to be expected if the molecular basis of action of cannabidiol (as anticonvulsant) and of THC (as psychotropic agent) are different.

Experimental Section

Cannabidiol was isolated from hashish.[28] Phenytoin was the product of Parke-Davis & Co. and was used without further purification. Me_2SO-d_6 (99.5%) and Me_2CO-d_6 (99.8%) were the product of Merck.

^1H and ^{13}C NMR were recorded on a Brucker WP-60 equipped with a FT attachment, operating at 60 MHz for ^1H and 15.08 MHz for ^{13}C NMR. The temperature was maintained within 1 °C by a variable-temperature control instrument. The ^{13}C NMR spectrum is the result of 130 000 scans with decoupling.

Acknowledgment. We thank the National Institute on Drug Abuse for generous support and Dr. O. Kennard, Cambridge, U.K., for helpful correspondence.

(25) G. C. Levy and G. L. Nelson, "^{13}C NMR for Organic Chemistry", Wiley-Interscience, New York, 1972, p 24.
(26) Reference 1, p 120.
(27) R. Mechoulam and E. A. Carlini, unpublished observations.
(28) Y. Gaoni and R. Mechoulam, *J. Am. Chem. Soc.*, **93**, 217 (1971).

Book Reviews

Heparin: Structure, Cellular Functions, and Clinical Applications. Edited by N. M. McDuffie. Academic Press, New York. 1979. xxi + 387 pp. 16 × 23.5 cm. $19.50.

The International Symposium on Heparin which was held in Saskatoon, Saskatchewan, Canada, in July 1977 is the basis of this book. The book is a compilation of 23 papers presented at this symposium. The major reasons for the symposium were to honor renowned heparin researcher Professor Louis B. Jaques and to enumerate the recent heparin research findings from around the world.

The book is organized into four sections, containing 22 papers and concluding remarks by Professor Jaques on "40 Years of Heparin Research—Past and Future". The four sections are "Structure", "Structure and Pharmacodynamics", "Cellular Function", and "Clinical Application".

The "Structure" section contains seven papers devoted to the structural features of heparin. Examples are "Enzymatic Degradation of Heparin as a Tool for Structural Analysis", "The Metabolism of Macromolecular Heparin", and "Structural Characteristics of Heparins revealed by Electrofocusing". Various chemical and enzymatic methods of structural elucidation are provided. For example, proton magnetic resonance spectra of heparins from different sources are examined and interpreted. Thin-layer chromatography, enzyme studies, and electrophoresis are also described. In this section, the reader will be able to learn the most recent research advances in heparin structural elucidation and recent advances in heparin chemistry. Medicinal chemists, biochemists, molecular biologists, and others interested in heparin's structure and activity will find this section of interest. I believe the reader will find the "Discussion" of these papers particularly stimulating.

In the section entitled "Structure and Pharmacodynamics", six papers describe various aspects of heparin's chemical structure in relation to its absorption, biological effects, interaction with factor VIII, and other substances like fibrinogen. The discussion of the chemistry of heparin binding sites requiring a specific sequence of different disaccharide units in the chapter entitled "Structural Basis for the Biological Effects of Heparin" by U. Lindahl will be of interest to medicinal chemists involved in drug design and synthesis.

The third division of the book entitled "Cellular Function" contains six papers. This section provides recent findings related to the functional role of heparin at the cellular level, comparison of heparin to chondroitin sulfate, hyaluronic acid and glycoproteins of nervous tissue, heparin's involvement in the storage of histamine in mast cells and its relationship to endothelium, and the pharmacological action of small doses of heparin.

The fourth division of the book entitled "Clinical Application" describes "Heparin Therapy in Venous Thrombosis and Pulmonary Embolism: Clinical and Experimental Observation", "Heparin in Inhalation", and "Clinical Use of Heparin and He-