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STUDIES OF CONFORMATION AND INTERACTION OF THE CYCLOHEXENONE AND ACETYL GROUP OF PROGESTERONE WITH LIPOSOMES

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Summary—The conformations of the A-ring and the 17-acetyl groups of progesterone were examined within liposomes, which were prepared from L-α-phosphatidylcholine in the presence or absence of cholesterol in the buffer, using qualitative nuclear magnetic resonance and circular dichroism of the progesterone spectra in the wavelength regions of 260–360 nm. The preferred conformational assignments, in the rotational conformations of the 17-acetyl group and invertible conformations of the cyclohexenone of progesterone were discussed on the basis of the elliptical strength of the Cotton effect and an energy estimation of the preferred conformers. Energetically unstable conformers of the acetyl group and α,β-unsaturated cyclohexenone of progesterone remarkably increased with an increase in the concentration of the liposomes. The liposomes containing 10% cholesterol were similar to the effect of the liposomes lacking cholesterol on the 17-acetyl group and the α,β-unsaturated cyclohexenone but those containing 50% cholesterol showed an increase in the number of energetically stable conformers of the α,β-unsaturated cyclohexenone. The nuclear magnetic resonance signal from liposomes together with the progesterone indicated the existence of the progesterone adjacent to a double bond or ester moiety in the lipid molecule. Therefore, it was apparent that the liposomes and the cholesterol within the liposomes regulated the conformational populations of both the cyclohexone and acetyl groups of the progesterone molecule.

INTRODUCTION

The two-step model of steroid hormone action described initially by Jensen *et al.* [1], in which steroids diffuse freely across cell membranes and carry out their action at the level of the cell nucleus, where they interact with receptors which then bind DNA, is not sufficient to account for all the known effects of steroids. Baulieu *et al.* [2] have demonstrated that the PROG and other steroid molecules are able to promote the maturation of *Xenopus laevis* oocytes through interaction with the cell membrane. The PROG also strongly inhibits hemolysis of human erythrocytes due to osmotic shock within several minutes of exposure to the agent [3]. Liver glycogen phosphorylase is early activated by several steroid hormones [4–7], this prompt glycogenolytic effect is independent of activation of protein synthesis by these steroids. Moreover, studies on androgens [8] have led to the conclusion that the activity of these hormones involves interaction with cell membranes. It thus appears that, in addition to the receptor-mediated mechanism, steroids may also operate through other mechanisms and, in particular, through an effect on the plasma membrane.

Studies with spin-labelled steroids in lecithin model membranes [9] and biomembranes from Leydig cell tumours [10] have indicated that steroids interact with biomembranes mainly through phospholipids. Moreover, the different steroids act at different levels of the fatty acyl chains in the lipid bilayer [11] and therefore this interaction would be highly specific [12–14].

NMR spectroscopy is one of the most powerful methods for studying the interaction between membrane and steroids. NMR is the nonperturbing spectroscopic technique most used to obtain static and dynamic structural information on membranes. For example, recent investigations using ^{2}H-, ^{13}C- and ^{1}H-NMR have been carried out to study the steroid–membrane

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Abbreviations: PROG, progesterone; CHO, cholesterol; PC, L-α-phosphatidylcholine: NMR, nuclear magnetic resonance; CD, circular dichroism.

interaction [15–17]. However, although CD appears to be more suitable for monitoring the conformational alteration of optically active compounds, it has not been used for investigation of interaction between steroids and membranes except for measurements of steroid–lipid [18] and steroid–protein [19, 20] interactions in organic solvents.

The aim of this study was to obtain more detailed molecular information of interaction of the PROG within the phospholipid bilayer of the model membrane. We used small unilamellar vesicles because the phenomena of differential light scattering and absorption flattening are not significant in them [21].

MATERIALS AND METHODS

Reagents

PC (egg yolk, type V-E), hydrocortisone-21-hemisuccinate and PROG were purchased from Sigma. The CHO was obtained from Nakarai Chemicals Ltd. All chemicals were used without further purification.

CD measurements

Vesicle dispersions were prepared by dissolving weighed amounts of PC, or mixtures of PC with CHO, in chloroform and then removing the solvent under an N_2 atmosphere and in vacuum. The appropriate buffer (Tris-HCl, pH 7.5, 5 mM) was added to give the desired concentration and the sample was quickly vortexed, then sonicated to clearness in a ice/water bath. The hormones were prepared daily with the buffer and added to the vesicles 30 min before the spectra were recorded. Ethanol was first added to solubilize the PROG powder but its final concentration was always 0.5%.

The CD measurements were performed at room temperature with a Jonbin–Yvon Mark III dichrograph calibrated with (+)-D-camphorsulphonic acid and equipped with a computerized data processing system. For the experiments, the spectra of the PROG were made in a quartz cell with a 10 mm path length, using a full scale deflection of 0.02 and a spectral bandwidth of 2 nm. The spectra in each figure have been recorded within the same day on the same chart. Results were expressed as molar ellipticity, θ (degrees/cm^2/dmol), calculated with reference to the PROG concentration. The background due to the dichroic scattering of the vesicles in all the spectra of CD has been subtracted.

NMR measurement

Vesicles dispersion was prepared by dissolving weighed amounts of PC or mixtures of PC with the cortisol or the PROG in methanol and then removing the solvent under N_2 and in vacuum ^2H_2O was added and sonicated for 30 min in an ice/water bath. ^{1}H-NMR experiments were carried out on a Varian XL-200 (200 MHz) spectrometer, ^{1}H-NMR chemical shifts were referenced to an internal standard of 3-(trimethysilyl)-propane sulphonate. All NMR experiments were performed at room temperature.

RESULTS

The CD spectrum of PROG (50 μM) in Tris-HCl 5 mM at pH 7.5 is shown in Fig. 1. The CD curve showed a positive Cotton effect at 275 nm due to n–π^* transition of the saturated ketone and a negative one at 315 nm due to π–π^* transition of α,β-unsaturated ketone. These results were in accord with previous works [18, 22], although using different solvents. The CD spectra of PROG in the presence of two concentrations of PC liposomes are shown in the same figure. The shapes of the spectra are similar to the spectrum of PROG above but the positive ellipticity corresponding to the acetyl group decreases with the increase of PC concentration in the liposomes for all bands. There is a remarkable decrease of the positive

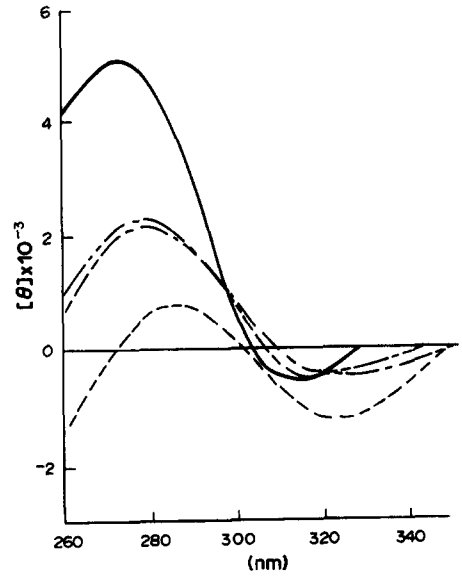

Fig. 1. CD spectra for 50 μM of PROG in the presence of various concentrations of PC liposomes. 0 mg/ml (——), 0.5 mg/ml (– – – –), 1 mg/ml (— — —), 2 mg/ml (— — —).

band in liposomes containing 2 mg/ml of PC. The interactions between PROG and the liposomes produced a shift in the PROG spectra towards higher wavelengths, which increased with the concentration of the PC. The negative bands corresponding to α,β-unsaturated cyclohexenone became wider in the presence of 0.5 and 1 mg/ml PC, but there was no change in the elliptical strength. However, the negative band markedly increased in strength when the PC concentration in the liposomes was 2 mg/ml.

Since cellular membranes contain the CHO, which has a similar structure to the steroid hormones, we examined the interaction between the PROG and PC liposomes containing CHO. Figure 2 shows the CD spectra of PROG in the presence of two different concentrations of the CHO in the liposomes. The positive band was shifted to higher wavelengths in the presence of CHO. Thus, the positive bands of PROG are scarcely influenced by the addition of CHO. However, the ellipticity of the spectrum of α,β-unsaturated cyclohexenone was modified when CHO was added to preformed liposomes. The negative band corresponding to α,β-unsaturated cyclohexenone of PROG was shifted to higher wavelengths when the CHO concentration of the PC liposomes was 10% and turned to a positive one in the presence of PC liposomes containing of CHO at 50%, as shown in Fig. 2. It was quite different in comparison with the curve obtained in the absence of CHO.

Table 1 shows the chemical shifts of the ^{1}H-NMR of PC in the presence or absence of the PROG and cortisol hemisuccinate. The assignments of the PC protons followed mainly those of Hauser *et al.* [23] and Kuroda and Fujiwara [24]. The most prominent changes in the chemical shifts were for the signals due to α-CH_2 and =C—CH_2—C= protons in the PC–cortisol system, and β-CH_2 and =C—CH_2—C= protons in the PC–PROG system. The differences of the chemical shifts of β-CH_2 and =C—CH_2—C= in the presence or absence of PROG were 0.18 and 0.05, respectively, and those of α-CH_2 and =C—CH_2—C= in the presence or absence of cortisol hemisuccinate were 0.04 and 0.07 ppm, respectively.

DISCUSSION

Advances in both CD instrumentation and the interpretation of ellipticity have reached a stage at which circular dichroism has become very useful as a probe in the conformational

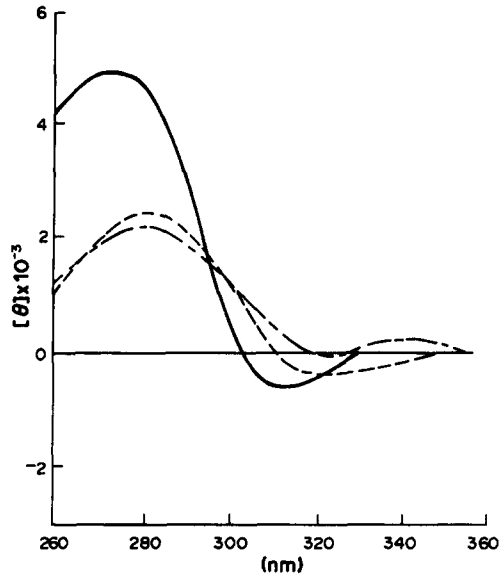

Fig. 2. CD spectra for 50 μM of PROG (——) and in the presence of PC liposomes (1 mg/ml) containing CHO: 10% (– – –), 50% (— — —).

analysis of molecules as well as in the study of interaction between proteins and steroids [19, 20]. CD is also a convenient and efficient technique for preferred conformational analysis of the acetyl group [25–27] and α,β-unsaturated cyclohexenone [28] of steroids. The asymmetric conformation around the chromophore of the acetyl group and cyclohexenone is changed by interaction, which theoretically can lead to differences in the Cotton effects of PROG, giving rise to negative and positive Cotton effects at 330 and 288 nm attributable to cyclohexenone and the acetyl group, respectively. The rotational strength of these Cotton effects shows an increase or decrease depending on conformational changes. Changes in strength of the CD curve in the presence or absence of CHO in the liposomes make it possible to study the specific events taking place in the acetyl group

Table 1. Assignment and chemical shifts of PC protons of PC (30 mM), PC–cortisol (3:2) and PC–progesterone (3:2) vesicles

Assignment	Chemical shifts (ppm)		
Proton of PC	PC	PC–cortisol	PC–progesterone
CH_3	0.88	0.89	0.89
$(CH_2)n$	1.27	1.28	1.28
β-CH_2	1.55	1.55	1.73
CH_2C=C	2.03	2.04	2.03
α-CH_2	2.37	2.41	2.37
=C—CH_2—C=	2.81	2.89	2.86
$N(CH_3)_3$	3.24	3.25	3.25
CH_2N^+	3.70	3.70	3.70
O_3POCH_2	4.29	4.30	4.29
CH_2OCO	4.45	4.46	4.46
H^2HO	4.75	4.76	4.76
CH=CH	5.31	5.31	5.31

DS± 0.01 ppm.

and the α,β-unsaturated cyclohexenone of the PROG.

In conformational analysis of the PROG interacting within the liposomes, it is necessary to consider three factors; inversions of the cyclohexenone ring, inversions of the B, C and D rings, and a rotation of the acetyl group. Throughout the following discussion of conformational analyses, however, the conformation of the alicyclic moiety, except for that of the A-ring of the compound is assumed to be unchangeable because of its rigid structure. Thus, only the conformations of cyclohexenone and the acetyl group were studied by CD. With regard to conformational analysis of the acetyl group, we must consider the problem of the bond joining of a carbonyl group to a tetrahedral carbon in a molecule of the acetone type in the rotational barrier. As can be seen in Fig. 3, the energy curve of the rotational acetyl group was estimated by using the energy function given for calculation of the energy of interactions for α-acetylcyclohexanols [18, 29] and pregnan 20-one [30], where θ corresponds to a dihedral angle [31] of the Newman projection [32]. The sign of the Cotton effect shown was predicted according to the octant rule [33] by examination of the model [31] for each conformer. Conformers with dihedral angles of 0–60° and 240–360° should exhibit a negative Cotton effect, while those angles of 60–240° should display a positive one. Judging from the energy of interactions, the conformers with dihedral angles of approx. 120–240° may be relatively preferred for the acetyl group of the compound (Fig. 3). Needless to say, other factors, such as temperature and solvents, should also affect the preference of rotational conformation of the acetyl group. The CD curves caused by the absorption of the acetyl group of PROG showed only a positive Cotton effect, suggesting that the predominant conformers were $\theta = 120$–$240°$ in the buffer although the elliptical strength of the curves was evidently dependent on the liposomes' concentration as shown in Fig. 1. The directionality of decreases in the band was distinctly regulated by the liposomes. The interactions between the acetyl group and the liposomes of 0.5 and 1 mg/ml induced a remarkable decrease in the ellipticity and evenly dominated the conformation of the acetyl group. However, the highest concentration of the liposomes strongly influenced the increase in populations of the energetically unstable conformers with dihedral angles of 0–60° and 240–360° than 120–240° which predicted the positive Cotton effect (Fig. 3). It was concluded that the liposomes induced the energetic unstable conformers of the acetyl group of the PROG. The CHO in the liposomes contributes a little to conformational change in the acetyl group of the PROG. That is to say, the energetically unstable conformers with the dihedral angles of 0–60° and 240–360°, with a negative Cotton effect, became predominant as a result of interaction between the acetyl group and the liposomes regardless of the CHO.

On the other hand, three conformers and predictable signs of the Cotton effect of the α,β-unsaturated ketone moiety of PROG are shown in Fig. 4. The conformer Ba predicts a negative sign while the conformers Bb and Bc predict a positive one according to the α,β-unsaturated octant rule [28] by examination of the Dreiding model [31]. Therefore, the negative Cotton effect of PROG observed at the wavelength of 315 nm should be assignable to absorption of the conformer Ba. The α,β-unsaturated A-ring absorption of the PROG was not affected by 0.5 and 1 mg/ml of the PC liposomes (Fig. 1) and by 10% of CHO in the

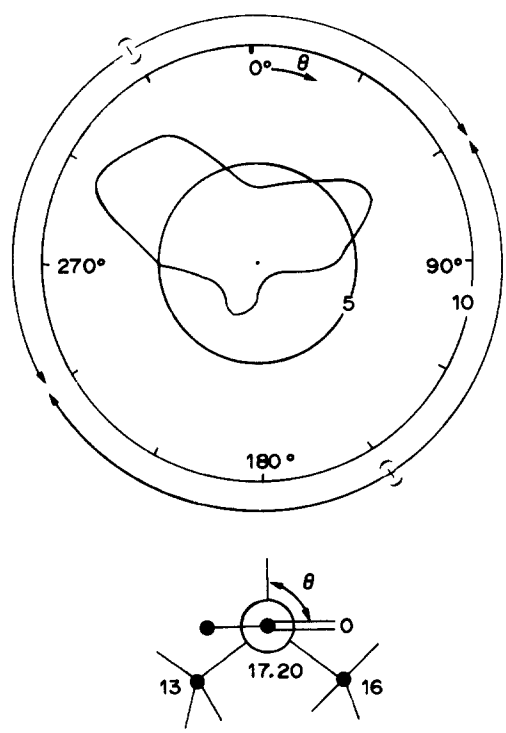

Fig. 3. Energy curve of interactions calculated for the rotational conformations of the acetyl group of PROG as a function of θ which indicates the dihedral angle as viewed from C_{17} to C_{20}. The predicted signs of the Cotton effect presented by the Newman projection are shown on the outer circle, and the inner and middle circles represent 5 and 10 kcal/mol lines, respectively.

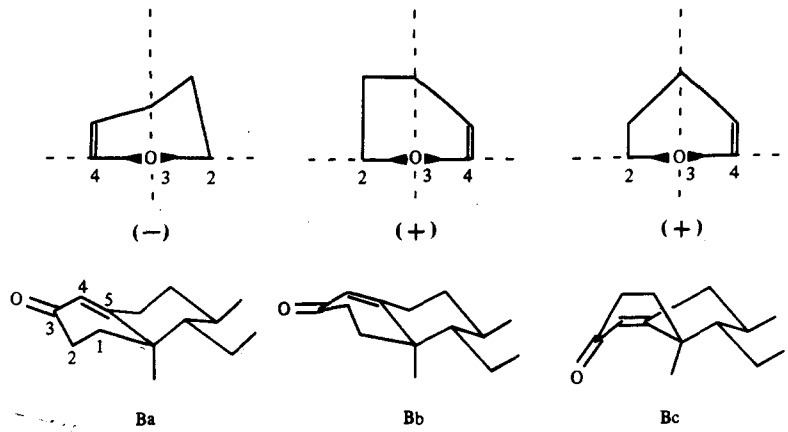

Fig. 4. Possible preferred conformations of the α,β-unsaturated cyclohexenone ring of PROG and predicted signs of the Cotton effect as viewed from the oxygen of the carboxyl group to carbon C_3.

liposomes (Fig. 2). This negative absorption, however, sharply increased with the increase in the concentration (2 mg/ml) of PC liposomes in the buffer and decreased in the presence of 50% of CHO in the liposomes (Fig. 2). The decrease in the negative elliptical strength was caused by the CHO, which stabilized the architectures of the liposomes or caused fluidization of the bilayer in the liposomes. Since the introduced CHO within the liposomes is familiar to the hydrocarbon moiety in the lipids because of hydrophobic property, it might be forced to move a part of PROG in the moiety toward the ester bonds in the lipids. This movement of PROG serves to induce special conformations due to electrostatic interactions between the ester and the α,β-unsaturated ketone. The conformer Ba in Fig. 4, with five carbons of C_2, C_3, C_4, C_5 and C_{10} on the same plane, probably shifts to an equilibrium containing Bb and/or Bc as a result of the interaction of pi electrons localized on the double bonds in the cyclohexenone of the A-ring with those on the carbonyl group contained in the ester moiety. Furthermore, the appearance of the positive rotational strength of PROG in the presence of CHO might be due to the increase in the population of the stable conformer Bb, but not to the conformer Bc. The Bc form may be slightly more unstable than the half-chair conformer Bb which neglects the interaction of ^{19}Me and C=O as can be predicted in the case of the Bc form with carbons of C_1, C_2, C_4 and C_5 on the same plane. In addition, the half-chair conformer Bb is about 1.6 kcal/mol [34] more energetically stable than the boat conformer Bc. The chemical shifts differences of 0.18 ppm in β-CH_2 and of 0.05 ppm in =C—CH_2—C= between PC liposomes and PC liposomes-PROG were based on an anisotropic effect which was induced by electron circulations on the double bonds in α,β-unsaturated ketone. The shifts of the β-CH_2 and the =C—CH_2—C= to a lower magnetic field by PROG showed that PROG was localized within the liposomes. Two groups of the —CH_2 and the =C—CH_2—C= were located in a plane which was formed by five carbons of C_2, C_3, C_4, C_5 and C_{10}. Therefore, PROG exists in a space of two hydrocarbon chains of one lipid molecule. Specially, the observation of the chemical shift difference in the =C—CH_2—C= showed the fact that the PROG exists in inner space because double bonds in the hydrocarbon moiety in the lipid are far from a polar part of the lipid. Except for two groups of the β-CH_2 and the =C—CH_2—C=, no chemical shift difference was observed in the spectra for the liposomes and the liposomes-PROG, as well as phosphorous moiety as shown in Table 1. It is clear from NMR and CD results that PROG is located in a relatively restrictive place in the lipid molecule and that there are some specific conformations in the acetyl group and the α,β-unsaturated ketone of the steroid. On the other hand, NMR spectra of the liposomes in the presence of cortisol hemisuccinate, which has different substitutions at the C and D rings from PROG, showed quite similar chemical shifts to liposomes-PROG except for the α-CH_2 and β-CH_2. The observation of the chemical shift difference of the α-CH_2 in the presence of the cortisol showed that cortisol hemisuccinate with bulky substitutions could not so closely approach the α-CH_2 moiety of lipid molecules in liposomes.

The results obtained here present new insights into the effect of the liposome environment on

the conformations of both the rotational acetyl group and the inversional cyclohexenone of PROG. Specifically, the conformational modification is not only influenced by the liposomes, but also by the CHO contained in the liposomes. These factors also characteristically regulate the distribution of the preferred conformations of PROG towards stable or unstable positions. The increase of unstable conformers, both by the liposomes and by CHO, might be the first step of the action of the steroid because these energetic conformers should become the stable conformers to transfer the energy to materials in the membrane. A part of the biological signals might reach final response through a transfer of the energy on the basis of the conformational changes.

Biomembranes are composed of many kinds of lipids with great variations with respect to structure, components and quantity. Some of the moieties of the lipids of biomembranes are forced into a particular conformation by the acetyl group and the cyclohexenone ring of PROG. The particular conformations which may be predominant in a population, as well as the basal lipids which make up the membrane architecture, may affect specific interaction with other components, such as various enzymes with special conformation. Indeed, it has been suggested that the membrane enzymes or receptors are unable to function properly unless they are surrounded by an appropriate phospholipid for the requirement of pertinent conformation [35–37]. Membrane enzymes which interact with PROG might undergo a conformational change accompanied by a modification in their function. This may cause functional modifications and may provide a reasonable explanation for the nongenomic effect of the steroid.

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REFERENCES

1. Jensen E. V., Suzuki T., Kawashima T., Stumpf W. E., Junsblut P. W. and De Sombre E. R.: A two-step mechanism for the interaction of estradiol with rat uterus. *Proc. Natl Acad. Sci. U.S.A.* **59** (1968) 632–638.
2. Baulieu E. E., Godeau F., Schorderet M. and Schorderet-Slatkine S.: Steroid-induced meiotic division in *Xenopus laevis* oocytes: surface and calcium. *Nature* **275** (1978) 593–598.
3. Kaya H. and Saito T.: Effect of progesterone and its 17α-hydroxyl derivative on human erythrocyte membrane. *Japan J. Pharmac.* **39** (1985) 299–306.
4. Egaña M., Sancho M. J. and Macarulla J. M.: An early effect of cortisol, previous to its glycogenogenic action. *Horm. Metab. Res.* **13** (1981) 609–611.
5. Diez A., Sancho M. J., Egaña M., Trueba M., Marino A. and Macarulla J. M.: An interaction of testosterone with cell membranes. *Horm. Metab. Res.* **16** (1984) 475–477.
6. Sanchez-Bueno A., Sancho M. J., Trueba M. and Machrulla J. M.: An early effect of estradiol at hepatic level, previous to its protein synthesis activation. *Int. J. Biochem.* **19** (1987) 93–96.
7. Sancho M. J., Gomez-Muñoz A., Sanchez-Bueno A., Trueba M. and Marino A.: Glycogen phosphorylase activation by progesterone in liver. *Exp. Clin. Endocr.* **92** (1988) 154–160.
8. Blyth C. A., Freedman R. B. and Robin B. R.: Sex specific binding of steroid hormones to microsomal membranes of rat liver. *Nature* **230** (1971) 137–139.
9. Sackmann E. and Trauble H.: Studies of the crystalline–liquid crystalline phase transition of lipid model membranes. II. Analysis of electron spin resonance spectra of steroid labels incorporated into lipid membranes. *J. Am. Chem. Soc.* **94** (1972) 4492–4498.
10. Sato B., Huseby R. A., Matsumoto K. and Samuels L. T.: Molecular nature of interaction of steroids with biomembranes related to androgen biosynthesis. *J. Steroid Biochem.* **11** (1979) 1353–1359.
11. Vincent M., Gallay J., De Foresta B. and Alfsen A.: *Liposomes in the Study of Drug Activity and Immunocompetent Cell Functions.* Academic Press, New York (1980) p. 1970.
12. Gallay J., Vincent M., De Paillerets C., Rogard M. and Alfsen A.: Relationship between the activity of the 3-hydroxysteroid dehydrogenase from bovine adrenal cortex microsome and membrane structure. Influence of proteins and steroid substrates on lipid "microviscosity". *J. Biol. Chem.* **256** (1981) 1235–1241.
13. De Paillerets C., Gallay J., Vincent M., Roeard M. and Alfsen A.: Membrane lipid dynamic and enzymic activity in bovine adrenal cortex microsomes. *Biophys. Acta* **664** (1981) 134–142.
14. Gallay J., Vincent M. and Alfsen A.: Dynamic structure of bovine adrenal cortex microsomal membranes studied by time-resolved fluorescence anisotropy of all trans-1,6-diphenyl-1,3,5-hexatriene. *J. Biol. Chem.* **257** (1982) 4038–4041.
15. Makriyannis A. and Fesik S.: Mechanism of steroid anesthetic action: interactions of alphaxalone and Δ^{16}-alphaxalone with bilayer vesicles. *J. Med. Chem.* **26** (1983) 463–465.
16. Fesik S. and Makriyannis A.: Geometric requirements for membrane perturbation and anesthetic activity. Conformational analysis of alphaxalone and Δ^{16}-alphaxalone and ^{2}H NMR studies on their interactions with model membranes. *Mol. Pharmac.* **27** (1985) 624–629.
17. Makriyannis A., Siminovitch D. J., Gupta S. K. D. and Griffin R. G.: Studies on the interaction of anesthetic steroids with phosphatidylcholine using deuterium and carbon-13 solid state NMR. *Biochim. Biophys. Acta* **859** (1986) 49–55.
18. Watanabe S., Saito T. and Ikeda M.: Conformational study of the acetyl group and cyclohexenone in progesterone interacting with phosphatidyllipid by means of circular dichroism. *J. Steroid Biochem.* **31** (1988) 853–860.
19. Watanabe S.: Circular dichroism study of the interaction between conformationally altered human serum albumin and testosterone. *J. Steroid Biochem.* **23** (1985) 177–183.

20. Ronald T. and Gene F. L.: Circular dichroism of bovine serum albumin–testosterone system. *J. Steroid Biochem.* **7** (1975) 1523–1525.
21. Mao D. and Wallace B. A.: Differential light scattering and absorption flattening optical effects are minimal in the circular dichroism spectra of small unilamellar vesicles. *Biochemistry* **23** (1984) 2667–2573.
22. Gergely A., Szász G. and Soos J.: New method for the selective determination of corticosteroids by means of difference CD spectroscopy. *Fresenius Z. Anal. Chem.* **323** (1986) 157–161.
23. Hauser H., Phillips M. C., Levine B. A. and Williams R. J. P.: Ion-binding to phospholipids. Interaction of calcium and lanthanide ions with phosphatidylcholine (lecithin). *Eur. J. Biochem.* **58** (1975) 133–144.
24. Kuroda Y. and Fujiwara Y.: Locations and dynamical perturbations for lipids of cationic forms of procaine, tetracaine, and dibucaine in small unilamellar phosphatidylcholine vesicles as studied by nuclear Overhauser effects in ^{1}H nuclear magnetic resonance spectroscopy. *Biochim. Biophys. Acta* **903** (1987) 395–410.
25. Suga T. and Watanabe S.: Stereochemical studies of monoterpene compounds. XIII. The conformational analysis of the acetyl group of 4-methyl-10-nor-8 oxomenthols by circular dichroism. *Bull. Chem. Soc. Japan* **45** (1972) 240–245.
26. Snatzke G. and Schwinum E.: Circular dichroism. XVII. Low-temperature determinations on 12-substituted 20-oxopregnanes and -16-pregnenes. *Tetrahedron* **22** (1966) 761–771.
27. Wellman K. M. and Djerassi C.: Optical rotatory dispersion studies. XCVIII. The conformation of the 17-acetyl group in steroids and its correlation with variable-temperature circular dichroism. *J. Am. Chem. Soc.* **87** (1965) 60–66.
28. Snatzke G.: Circulardichroismus—IX. Modifizierung der Octantenregel für α,β-ungestättigte Ketone: Transoide Enone. *Tetrahedron* **21** (1965) 421–438.
29. Suga T., Shishibori T. and Matsuura T.: Stereochemical studies of monoterpene compounds. VI. Stereochemistry of 2-hydroxypinocamphore and its reduction products. *Bull. Chem. Soc. Japan* **41** (1978) 1175–1179.
30. Allinger N. L., Carabblé P. and Perez G.: Conformational analysis. XLIX. The conformations of the acetyl side chains of some compounds related to pregnan-20-one. *Tetrahedron* **22** (1966) 1615–1623.
31. Examined by "Dreiding Stereomodels" W. Buchi Manufacture of Glass Apparatus Flawil, Switzerland.
32. Eliel E. L., Allinger N. L., Angyal S. J. and Morrison G. A.: *Conformational Analysis.* Interscience Publishers, New York (1965) p. 52.
33. Moffit W., Woodward R. B., Moscowitz A., Klyne W. and Djerassi C.: Structure and optical rotatory dispersion of saturated ketones. *J. Am. Chem. Soc.* **83** (1961) 4013–4018.
34. Hendrickson J. B.: Molecular geometry. I. Machine computation of the common rings. *J. Am. Chem. Soc.* **83** (1961) 4537–4547.
35. Roleofsen B.: The (non)specificity in the lipid-requirement of calcium- and (sodium plus potassium)-transporting adenosine triphosphatases. *Life Sci.* **29** (1981) 2235–2247.
36. Fleming J. W. and Ross E. M.: Reconstitution of beta-adrenergic receptors into phospholipid vesicles: restoration of ^{125}I iodohydroxybenzylpindolol binding to digitonin-solubilized receptors. *J. Cyclic Nucl. Res.* **6** (1980) 407–419.
37. Loh H. H. and Low P. Y.: The role of membrane lipids in receptor mechanisms. *Ann. Rev. Pharmac. Toxic.* **20** (1980) 201–234.