Additional Conformational Data for the Mapping of the Progestin Binding Site: Crystal Structures of 21-(Phenylseleno)progesterone and 17α-(Phenylseleno)progesterone

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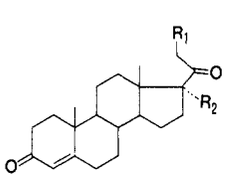
The crystal structures of 21-(phenylseleno)progesterone (1), which binds with moderate affinity to the progestin receptor, and 17α-(phenylseleno)progesterone (2), which binds hardly at all, have been studied in an attempt to explain these differences in affinity and to obtain further information on the location of the progestin receptor binding site with respect to the progesterone molecule. The crystal structures were refined by isotropic thermal approximation to R values of 0.082 for 1 and 0.084 for 2. The unusual 17β side-chain orientation of 2 with a C16–C17–C20–O20 torsion angle of +13° compared to −7° for progesterone would seem to preclude hydrogen bonding with the progestin receptor binding site and provides strong supporting evidence for the contention that this site is located above the β face of the molecule. Any rotation of the C21 methyl group into a more appropriate position is furthermore impeded by the presence of the 17α-phenylseleno substituent. On the other hand, some hydrogen bonding can occur in the case of 1 (C16–C17–C20–O20 = −31°) despite the fact that the difference in torsion angle (24°) with respect to progesterone is, in absolute values, greater than that for 2 (20°). This is because the orientation of the 17β-acetyl side chain of 1 is directed above the β face closer to the progestin binding site, as previously defined on the basis of data on a large number of molecules, and because the 21-phenylseleno substituent constitutes only limited steric hindrance to binding. Thus, the difference in affinity of these two compounds is entirely consistent with observations that the H-bond donor is located toward O20 in the β region of C16.

Amino acid sequence analyses of purified steroid receptor proteins are not yet available. Insight into the nature of the binding sites, with which steroid hormones interact in the cell, is thus generally obtained indirectly by correlating the molecular conformations of a wide variety of ligands with the corresponding receptor-binding data. If the ligands are sufficiently dissimilar, a mold of the volume occupied by their conformations might represent the binding site. However, much available data on the conformations of progestins[1-5] yield only limited information on the binding site, presumably because most progestins differ only by the nature and orientation of substituents partaking in 2, der Waals interactions rather than in hydrogen bonds. Data on novel steroids[6,7] are needed for further insight, and two such molecules are 21- and 17α-(phenylseleno)progesterone (1 and 2, respectively).[8]



1 R_1 = SePh; R_2 = H
2 R_1 = H; R_2 = SePh

In a recent study,[8] biochemical screening of 1 [21-(phenylseleno)pregn-4-ene-3,20-dione] and 2 [17α-(phenylseleno)pregn-4-ene-3,20-dione] showed that, compared to progesterone, 1 maintained appreciable binding affinity for the cytosol progestin receptor, whereas 2 had lost this capacity. In the present paper, further biochemical results are given, and the crystal structures of 1 and 2 have been undertaken, on the one hand, to establish whether this difference in relative binding affinity (RBA) may not be the consequence of different orientations of the 17β side chain and of different degrees of steric hindrance from the bulky phenylseleno group, and, on the other hand, to obtain further experimental data for the mapping of the progestin binding site.[6,7]

Biochemical Results

The phenylselenium-substituted progesterone derivatives 1 and 2 were assayed for binding affinity to five steroid hormone receptors in a routine screening system,[9-11] and the relative binding affinity (RBA) in each case was determined. As shown in Table I, whereas 17α-(phenylseleno)progesterone (2) did not bind to any significant extent to any receptor, 21-(phenylseleno)progesterone (1) retained appreciable binding affinity for the progestin (PG) receptor. An analysis of the kinetics of binding of 1 to the PG receptor by comparing RBA's recorded after short and long incubation times (as previously described[11-13]) sug-

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Table I. Relative Binding Affinities for Steroid Hormone Receptors[a,b]

compound	ES (2 h, 0 °C)	PG (24 h, 0 °C)	AND (2 h, 0 °C)	MIN (30 min, 25 °C)	GLU (4 h, 0 °C)
progesterone	<0.1	100	5.5 ± 0.6 (3)	8.0 ± 1.0 (3)	0.2 ± 0.1 (5)
21-(phenylseleno)progesterone (1)	<0.1	59 ± 14 (4)	1.7 ± 0.2 (3)	2.1 ± 0.3 (3)	0.6 ± 0.3 (4)
17α-(phenylseleno)progesterone (2)	<0.1	2.1 ± 0.5 (5)	<0.1	0.2 ± 0.1 (3)	<0.1

[a] The RBA's of the natural hormones estradiol, progesterone, testosterone, and aldosterone and of dexamethasone are taken to be equal to 100. [b] ES, PG, AND, MIN, GLU = estrogen, progestin, androgen, mineralocorticoid, and glucocorticoid receptors, respectively. The RBA values are the means ± SEM of the number of determinations indicated in parentheses.

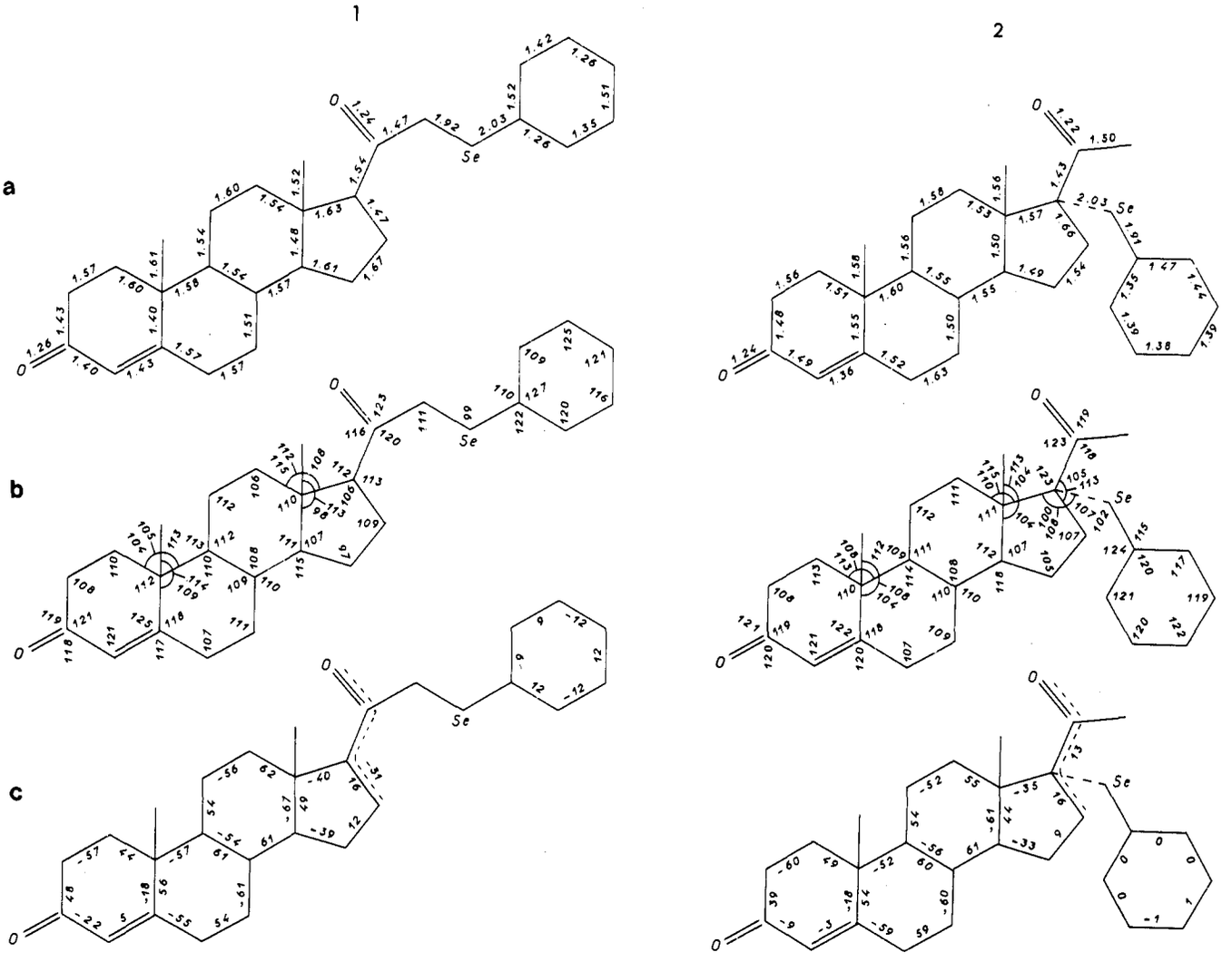

Figure 1. Geometrical characteristics of 1 and 2: (a) bond lengths, in angstroms; (b) valency angles, in degrees; and (c) dihedral angles, in degrees.

gested that the 1–PG receptor complex dissociates more slowly than the progesterone–PG receptor complex. Whereas the RBA of 1 was only 13 after 2 h incubation, it was 59 after 24 h incubation. This corresponds to an approximately fourfold increase with time. By definition, the RBA of progesterone, taken to be equal to 100 under each set of experimental conditions, remains unchanged. Thus, 1 forms a more stable, more slowly dissociating, complex with the receptor than does progesterone.

Crystal Structure Determinations. Compounds 1 and 2 crystallize as very thin needles of average quality. Both crystals are monoclinic $P2_1$, $Z = 2$, with $a = 14.55$ (1), $b = 7.26$ (1), $c = 11.30$ (1) Å, and $\beta = 100.8$ (1)° for 1, and $a = 17.61$ (1), $b = 7.35$ (1), $c = 8.99$ (1) Å, and $\beta = 97.9$ (1)° for 2. The structures were determined by direct methods and Fourier maps. They were refined with 816 independent reflections for 1 and 1521 reflections for 2. Because the crystals were small, only the selenium atom was refined with anisotropic thermal coefficients; the isotropic thermal approximation was used for the C and O atoms. Hydrogen atoms were not introduced. The final R and R_ω values are 0.082 and 0.089 for 1 and 0.084 and 0.096 for 2. The atomic coordinates are given as supplementary material (see paragraph at the end of paper concerning supplementary material).

The limited data in relation to the number of parameters to be refined could not disclose any specific features of the covalent bonds and of valency angles. For the C and O atoms, $\bar{\sigma}$ values are 0.030 Å, 3.5° for 1 and 0.025 Å, 2.5° for 2. The Se–C bonds are between 1.91 and 2.03 Å, and the C–Se–C angles are between 99 and 102° (Figure 1). This degree of precision was considered sufficient for the purpose of this study, i.e., for the comparison of the observed conformations of 1, 2, and progesterone (Figure 2)

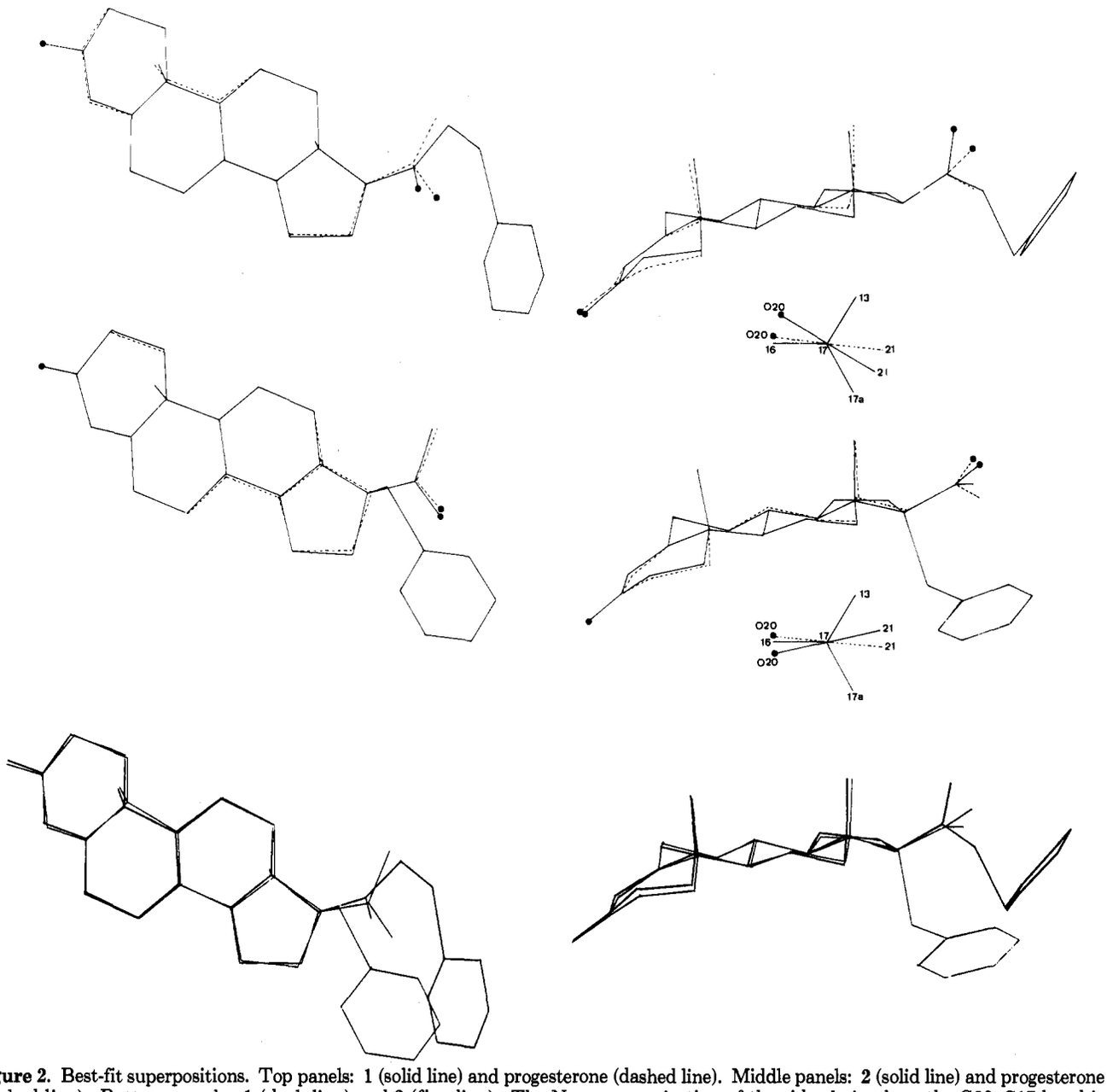

Figure 2. Best-fit superpositions. Top panels: 1 (solid line) and progesterone (dashed line). Middle panels: 2 (solid line) and progesterone (dashed line). Bottom panels: 1 (dark line) and 2 (fine line). The Newman projection of the side chain along the C20–C17 bond is shown.

in order to deduce structure–affinity relationships for mapping of the progestin receptor binding site.

The main interest of the two steroids lies in their overall conformation and in the orientation and bulk of their 17β side chain in comparison to progesterone. As shown by a best-fit superposition of 23 homologous C and O atoms,[14] the conformations of 1 and 2 are very similar to that of the form 1 conformer of progesterone[15] (Figure 2). The mean differences with progesterone are 0.17 Å for 1 and 0.10 Å for 2. The backbones of 1 and 2 do not differ significantly, although the C1–C2 ring A region may be slightly different. The greatest discrepancy occurs for the 17β side chain: 0.71 Å for O20 of 1 and 0.35 Å for C21 of 2. The orientation of this 17β side chain is characterized by a C16–C17–C20–O20 dihedral angle of −31° (±7°) for 1 and +13° (±5°) for 2 compared to −7° for progesterone. In the crystal, the geometry of this chain brings the center of the phenyl ring to 3.9 Å from O20 of 1 (a nonextended chain conformation) and to 4.3 Å from O20 of 2.

Relationships between Crystal Structure and Binding Affinity. Recent studies in which the RBA values of various steroids for the progestin receptor have been confronted with data on their observed conformations (crystalline) and/or calculated conformations (minimization of internal strain energies)[2] have enabled us to delimit a zone with respect to the steroid skeleton where the formation of hydrogen bonds would appear to favor progestin binding.[6,7] The O20 oxygen of progesterone and of many of its derivatives, the oxygen atom of several lactones, and the hydroxy group of norethindrone can all theoretically form hydrogen bonds within this zone.[6,7] The C16–C17–C20–O20 dihedral angle (−31°) of 1 lies in the range (centered around −25°) generally observed for 21-substituted steroids[16] and enables effective hydrogen bonding between O20 and the receptor binding site.[17] The

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fact that 1 retains appreciable binding affinity for the progestin receptor thus confirms that the region of the binding site corresponding to C21 is fairly large and can accommodate a bulky phenylselenium group. On the other hand, the orientation of the 17β side chain of 2 is somewhat different from previously observed orientations for steroids with a bulky 17α substituent. The C16–C17–C20–O20 dihedral angle of 17α-substituted steroids tends to be small (ca. −5°)[16] but, to our knowledge, never as small as noted here (+13°) for 2 (i.e., on the α side of ring D).

The virtual lack of affinity of 2 for the progestin receptor would thus seem to be explained by its two distinguishing features: a 17β side chain oriented beyond a zone previously shown to permit hydrogen bonding[6,7] and/or steric hindrance from the bulky 17α-selenophenyl substituent. The 17α position can accommodate fairly large substituents (e.g., an acetate group) without the binding affinity being greatly affected. For instance, under our experimental conditions, after 24-h incubation at 0 °C, medroxyprogesterone acetate has an RBA of 306 ± 25 and chlormadinone acetate an RBA of 321 ± 35.[10,11] However, the presence of a selenophenyl substituent in the 17α position, because of steric hindrance and because of the 90° orbitals of selenium, affects the orientation of the 17β-acetyl side chain to the extent that the ketone group can no longer form adequate hydrogen bonds with the progestin receptor. Inspection of a model reveals that the steric hindrance between C21 and the hydrogen atoms of the phenyl ring of the 17α substituent prevents the 17β side chain from attaining high or even moderately negative values for the C16–C17–C20–O20 dihedral angle. The introduction of the selenophenyl substituent into position 21 does not have such a drastic effect. Derivative 1 may still be able to form the requisite hydrogen bonds. Other explanations relating to, for example, the charge of the molecule, cannot, however, be entirely ignored.

Acknowledgment. The helpful collaboration of M. Fortin and G. Teutsch is gratefully acknowledged. We are indebted to Professor C. Djerassi for kindly supplying us with the two steroids.

Registry No. 1, 74136-99-5; 2, 74137-00-1; progesterone, 57-83-0.

Supplementary Material Available: Atomic coordinates of compounds 1 and 2 (1 page). Ordering information is given on any current masthead page.

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N-(Substituted-phenyl)-D-glycopyranosylamines and Their *O*-Acetyl Derivatives as Potential Modifiers of the Formation of Glycosaminoglycans[1]

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D-Arabinosyl, D-ribosyl, D-glucosyl, D-galactosyl, D-mannosyl, and L-rhamnosyl *N*-glycosides of *p*-aminobenzoic acid and their *O*-acetyl derivatives have been synthesized, and their ability to (a) inhibit the replication of cultured B16 melanoma cells and (b) modify the synthesis of glycosaminoglycans by these neoplastic cells have been evaluated. The most cytotoxic compound of the series was *N*-(*p*-carboxyphenyl)-2,3,4-tri-*O*-acetyl-D-arabinopyranosylamine (8), which produced 50% inhibition of cellular proliferation at a concentration of 4 μM; a number of other compounds were relatively cytotoxic, causing 50% inhibition of cell replication at levels of 18 to 49 μM. These effects were not due to modification of glycosaminoglycan biosynthesis, since these compounds were ineffective as inhibitors or initiators of the formation of these macromolecules.

The sodium salt of *p*-aminobenzoic acid *N*-xyloside has been reported to be an effective inhibitor of the growth of a variety of transplanted rodent neoplasms.[3] This compound causes a number of biochemical changes in malignant cells, including modification of the metabolism of prostaglandins, cyclic nucleotides, phospholipids, and glycosaminoglycans (GAGS), and interference with amino acid and Ca^{2+} transport.[4] The action on GAGS is due to the capacity of this compound to serve as an artificial initiator of GAG synthesis, a process that results in the formation and secretion of GAGS of low molecular weight. Since alterations in the properties of GAGS may result in modification of the growth and metastatic patterns of malignant tumor cells, we have synthesized a variety of *N*-(substituted-phenyl)-D-xylopyranosylamines[5] and haloacetamidoalkyl β-D-xylopyranosides[6] and have demonstrated that members of these two series were cytotoxic

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