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The conformational flexibility of aromatic retinoids

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Abstract

AM1 and PM3 complete geometry optimizations were performed on 19 arotinoids congeneric with (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl] benzoic acid (TTNPB), a very potent agent in carcinoprevention and carcinotherapy. Sixteen TTNPB conformations with close energy were obtained and characterized; four representative conformations were then studied for 14 derivative compounds, for which we found a substantial non-planarity of the two aromatic moieties. Large rotational flexibility of the arotinoid ring fragments was predicted by both methods. Very low barriers (0.4–3.9 kcal/mol) were found for the tetralenyl ring rotation. The two methods also agreed in predicting benzoic acid moiety rotation in a wide range of torsion angle values except those close to 0 or 180 degrees for which the PM3 rotational barriers were found to be considerably lower than the AM1 ones. This high conformational flexibility of arotinoid molecules may facilitate their favorable orientation in the process of fitting to the receptor sites.

Key words: Arotinoids; Conformational analysis; Drug–receptor interaction; Geometry optimization; Rotational barriers

1. Introduction

Vitamin A (retinol) has long been recognized for its important effects on vision, on cell reproduction, and on differentiation of cells and tissues in culture. Retinoic acid (RA) has been identified as an activated metabolite of retinol *in vivo* and *in vitro*, a metabolite of beta-carotene *in vitro*, and a physiologically important supporter of vitamin A-dependent differentiation. The derivatives or analogues of vitamin A constitute an interesting family of biologically active compounds called *retinoids* [1,2].

These compounds reveal a broad spectrum of potential pharmacological applications. Of particular importance is their ability to control cellular differentiation and prevent excessive proliferation. These activities are responsible for the efficacy of retinoids in the prevention or suppression of carcinogenesis, in therapy for some neoplastic diseases, and in the treatment of some dermatological diseases such as psoriasis and possibly rheumatoid arthritis. Retinoids have been found to prevent cancer of the bladder, lung, and skin

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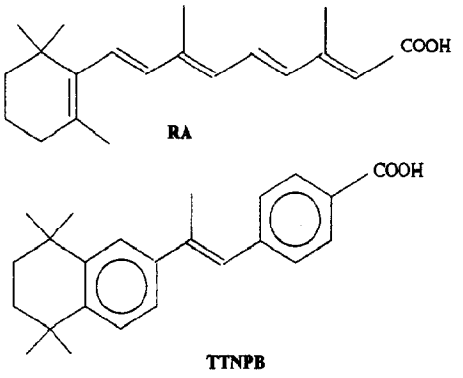

Fig. 1. Structures of retinoic acid (RA) and (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl] benzoic acid (TTNPB).

in experimental animals and to suppress malignant transformations. Certain retinoids have even shown the capacity to reverse the neoplastic transformation of cells [1–8].

The physiological importance of retinoids has led to the synthesis of several thousands of these compounds. The first synthetic retinoids preserved the basic structure of retinol and RA by retaining a lipophylic terminal ring, a polyenic side-chain (a spacer), and a polar terminal group. The ensuing generation of retinoids [9–13] which emerged after 1980, and is still expanding, has shown activity thousands of times higher than that of RA. The side-chain in these molecules is transformed into one or more aromatic rings, wherefrom the name *aromatic retinoids* or *arotinoids* was adopted (Fig. 1).

Although the crystal structure of TTNPB is known [9,13], no studies have been reported on the conformations allowed and the barriers of internal rotations in it or other aromatic retinoids. (Two communications [14,15] do mention the use of molecular modeling algorithms ADAPT [16] and RANDOM-SEARCH [17], respectively, but no data are given on the conformations thus

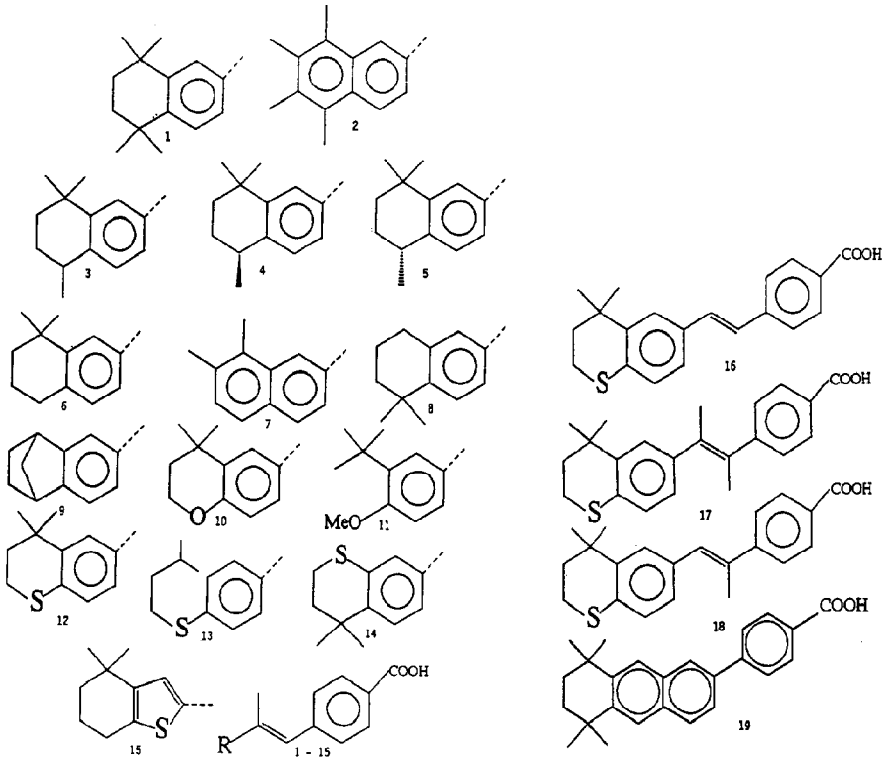

Fig. 2. The 19 aromatic retinoids under study (hydrogen atoms not shown). Compounds **1–15** are those from Table 18 of ref. [25].

modeled.) Indeed, the conformations of these molecules in solution or upon interaction with a receptor molecule may differ considerably. Any modeling of the potency of this important class of anticancer agents, however, must account for their conformational flexibility. To this end, in the study reported here, we aimed at elucidating the basic optimized geometry conformations, as well as the ranges of free rotations of the ring fragments, in a selected group of aromatic retinoids by using the AM1 (Austin Model 1) [18] and PM3 (MNDO Parametric Model 3) [19] semiempirical quantum chemical methods.

2. Methods and compounds

Owing to the relatively large size of the molecules of interest (typically about 50 atoms) we did not use *ab initio* calculations. The strength of *ab initio* methods is in characterizing electronic properties rather than in conformational analysis. Instead, we used two high-quality semiempirical methods, namely AM1 and PM3. These methods represent a major improvement over their predecessors MNDO [20] and MINDO/3 [21]. Thus, the overall errors in the heats of formation as calculated by AM1 and PM3, respectively, are about 40% and 65% less than those calculated by MNDO. Furthermore, both AM1 and PM3 produce rather reliable predictions for relative stabilities of various conformers.

The AMPAC (Austin Model Package) [22] was applied with the keyword PRECISE. All geometrical variables were optimized. The input coordinates and molecular images were prepared using the integrated OASIS package developed by the Laboratory of Mathematical Chemistry and Chemical informatics in Burgas, Bulgaria [23,24]. The OASIS technique includes 2D–3D conversion and preliminary structure optimization. The AM1 and PM3 conformational analyses were performed with 15 degree increments in the dihedral angles.

Fifteen arotinoids (Fig. 2) were selected for our analysis [25]. All contained a carboxylic group as a polar terminus and differed in the other terminal ring fragment. In addition, in order to investigate the stereochemical factors (the spacer methyl group(s)) influencing the ring rotations, the structure of compound **12** was modified so as to have either no methyl groups (compound **16**), two such groups (compound **17**), or a displaced methyl group (compound **18**). Compound **19** was also considered because it represents another potent arotinoid that, when compared to compounds **1–18**, is more conformationally restricted by an aromatic cyclization of a part of the spacer (Fig. 2).

The anticancer activities of compounds **1–15** and **19**, expressed as inhibitory doses (ID_{50}, nmol) required to inhibit by 50% the induction of ornithine decarboxylase assay (ODC) in mouse dorsal epidermis treated with the tumor promoter TPA [25], are listed here following the compound sequential order: 0.03, 1.6, 0.07, 0.05, 0.06, 1.1, > 170, 0.6, 3.1, 2.7, 0.4, 0.5, 170, 0.6, 0.1, and 0.07, respectively. The anticancer activity of compounds **16–18** has not been discussed in the literature. They have been chosen here as simple models for evaluating the steric effect of the spacer methyl group, as compared to compound **12**.

3. Results

3.1. Optimized geometries

The minimum energy geometries of TTNPB (Fig. 1), one of the most active retinoids known, were studied in detail. As seen in Fig. 3, the basic conformations are determined by the rotations of the two ring fragments. The latter are characterized by the torsion angles TA1 = C1–C2–C11–C12 and TA2 = C11–C12–C13–C14, respectively. Owing to the lack of symmetry between the left and right cyclic moieties, the four energy minima existing in each of them produce, in total, 16 conformations. The latter are characterized in Table 1 by the respective AM1 values of the two torsion angles of ring-rotation, as well as by their heats of formation and dipole moments.

The 16 conformations are presented in Table 1 in four groups, according to the signs of TA1 and

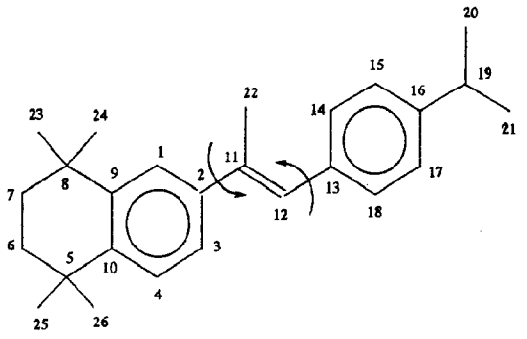

Fig. 3. The atom numbering in the molecular skeleton of TTNPB. The two cyclic fragment rotations are designated to occur about the 2–11 and 12–13 carbon–carbon bonds.

TA2 (+−, −+, −−, and ++, respectively). The difference in energy of these conformations ($\Delta\Delta H \leqslant 0.3$ kcal/mol) and in their dipole moments ($\Delta\mu \leqslant 0.5$ D) is very small. The most important feature of the equilibrium conformations is that, while the propenyl spacer remains in plane, both ring fragments deviate from the plane. In each of the four groups of conformations, three of the TA1 values are close to 40° or 140°, whereas the fourth one is near 50° or 130°. Similarly, for the right-cyclic-fragment deviation from planarity, three of each four conformations are described by TA2 near 44° or 136°, and the fourth one declines at about 58° or 122°. The third column in Table 1 shows the relative twist of the two-ring systems. Surprisingly, for half of the conformations the two-ring moieties were found to be twisted at 80–88°, i.e. to be almost orthogonal to each other, whereas the other half of the conformations were shown to have almost parallel rings (relative twist of about 2–5° only). However, as shown in Tables 2 and 3, the PM3 method produces qualitatively different results.

The AM1 and PM3 optimized bond lengths, valence angles, and torsion angles of the nonhydrogen atoms in conformation 1 of the TTNPB molecule are shown in Table 2. The two methods produced similar bond lengths and valence angles. In almost all cases the difference in the bond lengths was less than 0.01 Å. Most of the valence angles also differed by less than 1°, the exceptions being carbon atoms 7 and 8 (ΔVA = 2.5°), the four methyl carbons 23–26 (ΔVA = 1–2°), and the two oxygens (ΔVA = 1–1.3°). The discrepancies between the torsion angles, however, were considerable. The torsion angles of the carbons in the tetramethyl-substituted ring, as well as the four methyl carbon atoms themselves, differed from one another by 5–7°; for the two carboxylic oxygens this difference was already 13°. However, a striking discrepancy between the two methods was found for the basic torsion angle TA1 (C1–C2–C11–C12). The AM1 calculations showed the naphthalenic moiety to be about 51° out of plane, whereas the PM3 calculations specify it to be only 15°. This suggests that the two methods evaluated rather differently the steric interaction of the hydrophobic ring system and the propenylic methyl group. Since there was no such interaction for the benzoic acid ring, the AM1 and PM3 values of the other basic torsion angle TA2 (C11–C12–C13–C14) were very close.

Furthermore, the predicted relative twists of the two-ring fragments in conformation 1 of TTNPB were different: about 85° by AM1 versus only 56° by PM3. Both gas-phase predictions deviated equally from the X-ray result of 71° obtained for a solid-state conformation [9].

Table 1
The 16 conformations of compound 1: AM1 basic torsion angles, heats of formation, and dipole moments

Conformation	TA1	TA2	|TA1 + TA2| [a]	$-\Delta H$ (kcal/mol)	μ (D)
1	+129.1	−44.0	86	59.50	5.62
2	+141.2	−137.8	3.5	59.59	5.40
3	+39.5	−43.5	4	59.64	5.23
4	+40.3	−122.4	82	59.33	5.61
5	−139.7	+59.2	80.5	59.31	5.66
6	−140.7	+138.7	2	59.57	5.62
7	−39.1	+43.4	4	59.62	5.37
8	−50.2	+138.4	88	59.49	5.61
9	−140.7	−43.5	4	59.63	5.24
10	−140.1	−121.9	82	59.31	5.65
11	−50.3	−44.0	86	59.54	5.54
12	−39.1	−137.7	3	59.61	5.24
13	+141.2	+43.3	4.5	59.60	5.37
14	+129.1	+138.3	87.5	59.43	5.72
15	+41.1	+58.5	80.5	59.35	5.50
16	+39.4	+138.7	2	59.60	5.46

[a] Valid for conformations 1–8. For conformations 9–16, 180-|TA1+TA2| holds.

The comparison of the AM1 and PM3 calculations for TA1 and TA2 in TTNPB is extended in Table 3 to the whole series of 19 arotinoids. Four conformations are shown for each of compounds **1–14**, and one for each of compounds **15–19**. As seen in Table 3, the results for compound **1** and those for each of compounds **2–14** are basically the same. Thus, in the AM1 calculations the TA1 and TA2 values of these compounds varied within the very narrow ranges of 2.1° and 0.4°, respectively. These ranges, when obtained by the PM3 method, were only slightly larger: 3.2° and 0.6°. Evidently, due to the large interatomic distance, the changes in the structure of the nonaromatic hydrophobic ring did not cause any significant change in the torsion angles that determine ring-fragment nonplanarity. Hence, the result obtained for the relative twist of the two aromatic moieties in the TTNPB molecule held for each of compounds **1–14**: AM1 predicts two types of conformations in which the plane of the hydrophobic region and that of the acidic functionality were either nearly perpendicular or almost parallel to each other, whereas PM3 predicts configurations with these two regions twisted at angles close to 26° and 56°, respectively.

Compound **15**, however, does not share this feature. Both methods showed its pentadienyl ring to be almost in plane with the propenylic spacer, whereas the benzoic acid moiety is rotated at an angle similar to those in compounds **1–14**. As a result, the relative twist of the two rings in compound **15** was calculated to be 29° (AM1) and 37° (PM3) respectively. The geometries predicted by the two methods were also similar in compound **19**. Being conformationally more restricted than the other compounds in the series under study, compound **19** has only one basic torsion angle: TA1≡TA2. The relative twist of the two ring fragments, specified by this angle,

Table 2
AM1 versus PM3 optimized bond lengths, valence angles, and torsion angles, as well as atom-atom connectivities in TTNPB [a]

Atom	*R*	Alfa	Theta	Na	Nb	Nc
18						
13	1.4059/1.4012					
14	1.4014/1.3969	118.99/119.21				
15	1.3929/1.3897	120.53/120.50	−0.35/0.25	14	13	18
16	1.3994/1.3942	120.14/119.93	0.51/0.01	15	14	13
19	1.4732/1.4877	118.83/119.42	−179.56/−179.38	16	15	14
21	1.2320/1.2127	126.92/127.93	30.69/43.60	19	16	15
20	1.3687/1.3545	119.91/121.18	−148.83/−135.90	19	16	15
17	1.4005/1.3966	119.67/119.98	−0.64/−0.54	16	15	14
12	1.4534/1.4586	122.08/122.43	−178.34/−177.99	13	14	18
11	1.3476/1.3482	126.69/127.16	−43.97/−41.66	12	13	14
22	1.4826/1.4884	124.03/122.05	−0.33/−0.31	11	12	13
2	1.4679/1.4736	119.62/119.63	179.29/−179.89	11	12	13
1	1.3966/1.3947	121.12/121.35	129.06/165.54	2	11	12
9	1.4028/1.3972	121.58/121.95	180.00/179.89	1	2	11
10	1.4046/1.4002	119.25/119.32	1.44/0.01	9	1	2
4	1.4039/1.3991	118.88/118.85	−2.09/0.20	10	9	1
3	1.3884/1.3841	121.47/121.32	1.60/−0.04	4	10	9
5	1.5054/1.5143	122.97/122.58	178.34/−178.23	10	9	1
6	1.5292/1.5346	111.27/110.41	−13.81/−18.68	5	10	9
7	1.5074/1.5166	112.34/109.96	44.23/49.80	6	5	10
8	1.5293/1.5347	112.38/110.01	−61.77/−67.83	7	6	5
23	1.5285/1.5289	107.39/108.88	165.27/171.34	8	7	6
24	1.5261/1.5320	109.88/109.15	−76.72/−69.11	8	7	6
25	1.5262/1.5322	109.19/108.23	107.67/100.77	5	10	9
26	1.5285/1.5288	110.35/110.49	−132.99/−139.30	5	10	9

[a] Conformation **1** from Table 1 only. The atom numbering is that from Fig. 3; Only data for nonhydrogen atoms are shown.

was found to be near 41° (AM1) and 48° (PM3), respectively. The same angles were found for biphenyl, the simplest molecule with two aromatic moieties, which we used as a standard for comparison. These results are in agreement with the experimental relative twist of more than 30°, as experimentally determined in gas phase.

As pointed out in the foregoing for compound **1**, the PM3 conformations of compounds **2–14** differed considerably from the AM1 ones in their basic torsion angle, TA1. PM3 predicted the left phenyl ring to be only 14–20° out of plane. In contrast, AM1 predicted it to be either 48–50° or 60–62° out of plane. Questions arose about the reliability of these substantial, yet very different torsion angles, which show little conjugation between the two aromatic moieties. Hence, we expanded the initial series of 15 compounds with compounds **16–19**. With this new set, a systematic evaluation of the effect of the spacer methyl group became possible: molecule **16** with no methyl group; molecules 12 and 18 with one such group located closer to, or respectively farther from, the naphthalenic moiety; molecule **17** with two methyl groups; and finally, molecule **19** with a partially restricted spacer, i.e. with less rotational freedom.

Eliminating the methyl group in molecule **16** reduces the ring repulsion. As a result, the PM3 method correctly predicted planarity, whereas the AM1 method, although displaying the same trend, failed to arrive at a planar molecule and specified each of the two aromatic moieties to be near 20° out of plane. The opposite trend emerges when a second methyl group is attached at C12 (compound **17**). The stronger steric effect makes the two rings go out of plane at about 60° (AM1) and 75° (PM3), respectively. When the methyl group is displaced from C11 to C12 (compound **18**) the effect on the left ring system remains approximately the same (TA1 ≈ 40°) as in compounds **1–14**. The repulsion of the benzoic acid ring, however, is stronger since its deviation from planarity increases from 43–44° to 50–52°.

Table 3
Basic torsion angles obtained after AM1 and PM3 geometry optimization for some conformations of retinoids 1–19 [a]

Compound No.	TA1	TA2	|TA1 + TA2| [b]
AM1			
1–14	+128.3 to +129.9	−43.7 to −44.1	84.2 to 86.2
	−128.4 to −129.9	+43.5 to +43.8	84.6 to 86.4
	−139.6 to −141.7	−43.4 to −43.7	4.7 to 5.1
	+139.6 to +141.7	+43.2 to +43.4	3.8 to 5.1
15	+16.0	−45.1	29.0
16	+159.9	−23.5	43.6
17	+119.7	−62.7	57.0
18	+138.7	−50.3	88.4
19 [c]	40.7	40.7	40.7
PM3			
1–14	+163.4 to +165.9	−41.5 to −41.8	55.6 to 58.1
	−163.3 to −166.4	+41.1 to +41.4	54.7 to 58.1
	−160.1 to −163.3	−44.5 to −45.0	24.6 to 28.3
	+160.2 to +163.1	+43.9 to +44.5	24.1 to 27.3
15	+4.9	−42.3	37.4
16	−179.8	−0.2	0.0
17	+104.6	−76.9	27.7
18	+141.2	−52.2	89.0
19 [c]	47.6	47.6	47.6

[a] The compound numbering is as that in Fig. 2. Conformations 1, 5, 9, and 13 (see Table 1) are given for compounds **1–14**.
[b] Or 180 − |TA1 + TA2|.
[c] TA1≡TA2 for compound **19**.

Besides its failure to reproduce the planarity of molecule **16**, the AM1 method also showed in compounds **12** and **17** a much higher relative twist of the two cyclic fragments than the PM3 method (85.3 and 57.7 versus 56.3 and 27.7, respectively). One may thus suppose that the AM1 method tends to overestimate the steric effect of the spacer methyl group on the two aromatic moieties, whereas the PM3 ring torsion angles seem more reliable. This conclusion also implies that the lack of significant ring conjugation in compounds **1–14**, as predicted by the PM3 method, is not an artifact but rather is in good agreement with the substantial relative twist of the two aromatic fragments (71°) found experimentally for TTNPB [9].

3.2. Heats of formation and dipole moments

Some information on the energy minima and dipole moments of the 19 arotinoids is given in Table 4. As can be seen, almost all conformations studied for compounds **1–14** differed in their heats of formation by only 0–0.2 kcal/mol; the two exceptions were compound **2** (AM1) and compound **14** (PM3),for which this range was extended to 0.7 kcal/mol. All the molecules studied had rather high dipole moments, ranging from 3.7 to 7.6 D, which suggests a role for nonspecific electrostatic interaction between the receptor and retinoid molecule. The AM1 dipole moments, which were considered more reliable than the PM3 ones [26], were generally higher than the latter. For most of the conformations of compounds **1–14**, dipole moments determined by either method varied within a 0.4 D range. However, for conformations of compounds 12 and 13, the predicted dipole moments differed by 2–3 D, thereby indicating a strong affect on their interaction with the receptor.

3.3. Conformational analysis

The internal ring rotations about the carbon–carbon bonds 2–11 and 12–13 in compounds **1–19** were studied by varying the respective torsion angles TA1 (C1–C2–C11–C12) and TA2 (C11–C12–C13–C14) by 15°. For brevity, these two

Table 4
Heat-of-formation and dipole-moment ranges for four conformations of retinoids **1–15** and one conformation of compounds **16–19** [a]

Compound No.	$-\Delta H$ (kcal/mol)		μ (D)	
	AM1	PM3	AM1	PM3
1	59.49–59.63	70.81–70.87	5.24–5.65	4.77–5.40
2	31.23–31.96	48.42–48.48	5.32–6.08	4.98–5.64
3	55.54–55.68	63.07–63.14	5.25–5.71	4.92–5.51
4	58.19–58.33	65.97–66.04	5.23–5.67	4.76–5.01
5	58.09–58.23	65.95–66.01	5.22–5.66	4.81–5.42
6	54.90–55.05	61.49–61.56	5.22–5.64	4.83–5.47
7	22.40–22.47	32.58–32.65	4.80–5.49	4.47–5.03
8	54.95–55.07	61.44–61.49	5.22–5.60	4.77–4.80
9	16.91–17.06	26.98–27.04	5.23–5.99	5.29–5.70
10	80.35–80.50	88.01–88.09	5.10–6.01	4.74–5.69
11	81.56–81.71	86.73–86.78	5.27–6.14	4.21–4.59
12	41.76–42.10	45.34–45.61	4.81–7.51	3.69–6.26
13	52.56–52.71	53.36–53.42	4.75–7.57	3.89–6.09
14	41.84–41.97	44.85–45.52	5.64–6.66	5.90–6.31
15	51.54	50.74	4.26	4.77
16	37.69	39.76	6.28	4.15
17	46.46	52.71	5.10	4.94
18	41.90	45.28	4.99	3.87
19	49.12	60.44	5.46	4.76

[a] The conformations are those given in Table 3.

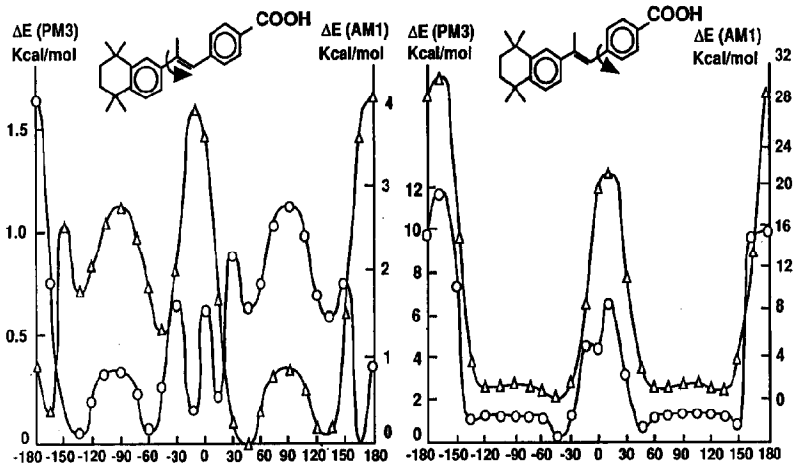

Fig. 4. (△) AM1 and (○) PM3 rotational curves for the TTNPB molecule. Energy barriers near 0°, ±90°, and 180°. Energy is relative to the global minimum set to zero.

cases will henceforth be termed 'left rotation' and 'right rotation', respectively. The optimized geometries of conformation **1** were used as starting geometries for compounds **1–14**.

The major result of our conformational analysis was that the molecules of the aromatic retinoids showed a large degree of freedom of internal ring rotations. This is illustrated by Figs. 4 and 5, where both types of ring rotations are shown for compounds **1** and **2**, as calculated by the AM1 and PM3 methods. Despite the large difference in their left ring systems (5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl versus 5,6,7,8-tetramethyl-2-naphthalenyl, both abbreviated henceforth as tetralenyl), the two molecules manifested quite similar behavior upon the two rotations. As seen in Figs. 4 and 5, both PM3 and AM1 rotational curves indicated practically no barrier for the left rotation. However, the two methods disagreed in predicting barriers for the

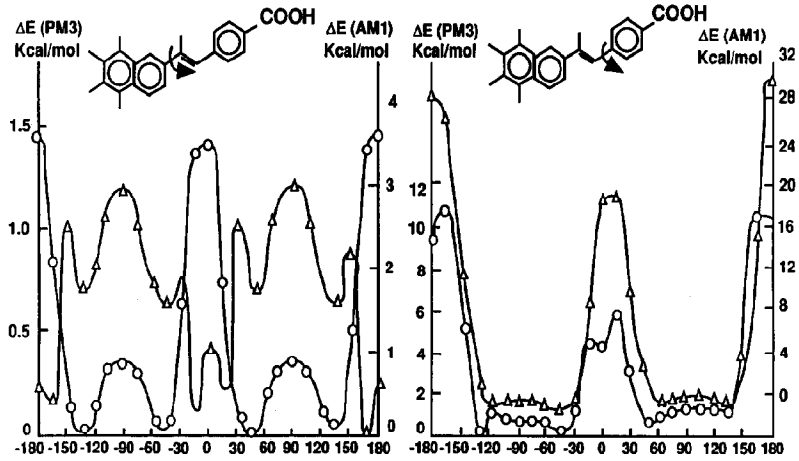

Fig. 5. (△) AM1 and (○) PM3 rotational curves for compound 2. Energy barriers near 0°, ±90°, and 180°. Energy is relative to the global minimum set to zero.

Table 5
AM1 and PM3 energy barriers for the rotations of the left and right cyclic fragments in the retinoids under study

Compound No.	Left rotation				Right rotation			
	PM3		AM1		PM3		AM1	
	±90	0; 180	+90	0; 180	15	−165	15	180 [a]
1–14	1.0–1.2	0.2–0.7	0.8–0.9	3.2–3.9	5.8–6.3	11.0–11.6	20.3–21.7	29.3–29.8
15	0.7	– [b]; –	1.1	–; –	5.2 [c]	10.7 [c]	23.7	32.7
					±90	0; 180	±90	0; 180
16	2.0	0.1; –	2.1	1.1; –	2.0	0.1; 0.3	1.9	0.2; 1.1
17	–	55.2; 56.2	0.1	65.5; 61.7	0.0	56.0; 54.3	0.1	64.3; 67.9
18	1.2; 1.3	10.3; 4.7	1.3; 1.4	29.6; 20.0	0.4	1.9; 1.8	0.9	3.6; 3.5
19 [d]	0.8	–; –	1.1	3.2				

[a] Angles in deg; compounds **2**, **7**, **9**, **11**, and **14** have energy barriers at −165° rather than at 180°.
[b] No energy maximum exists at angles denoted by a hyphen.
[c] −15 and +165°, respectively.
[d] One rotation only (TA1≡TA2).

right rotation. Distinct AM1 barriers were found near both 180° (almost 30 kcal/mol) and 0° (20–21 kcal/mol), thus preventing rotations within 20–30° around these energy maxima. The PM3 rotational barriers were considerably lower: about 6 kcal/mol near 0° and about 11 kcal/mol near 180°, thus leading to an equilibrium distribution of the different rotamers at room temperature.

As seen in Table 5, similar results were obtained for compounds **1–15**: practically no rotational barriers for the tetralenyl rings and contradictory AM1 versus PM3 predictions for the benzoic acid fragment rotation. The PM3 energy maxima of 5.8–6.3 kcal/mol at 15 degrees and those of 11.0–11.6 kcal/mol at −165° allow an equilibrium conformation distribution to be established at room temperature, whereas the AM1 barriers at 15° (20.3 to 21.7 kcal/mol) and near 180° (29.3 to 29.9 kcal/mol) are high enough and prevent such a result. This difference is even more pronounced for compound **15**, for which the PM3 barriers are even lower (5.2 and 10.7 kcal/mol) and the AM1 ones are higher (23.7 and 32.7 kcal/mol, respectively). On the other hand, due to the in-plane location of the left ring fragment, this compound has no barriers but energy minima at 0 and 180°.

The conformational analysis of compounds **15–18** also revealed other interesting features. For compound **18**, whose methyl group is shifted from C11 to C12 and interacts sterically with the right ring, the two methods predicted no barrier to the right rotation, as well as at ±90° left rotation; they however disagreed in describing the left rotation near 0 and 180°, AM1 showing barriers of 20–30 kcal/mol, but PM3 showing barriers of only 5–10 kcal/mol. For compound **17**, which has methyl groups at both C11 and C12, the two methods also agreed in showing very low energy maxima near ±90° and distinct maxima within a 54–68 kcal/mol range near 0 and 180°. A quite different pattern (free rotations with shallow, very low barriers) was exhibited by compound **16**, which is devoid of methyl substituents, as well as by compound **19**, whose two ring fragments are directly linked by a bond that thus specifies a single rotation only.

4. Conclusions

One may conclude that, despite the contradictory AM1 versus PM3 results for the rotational barriers of the benzoic acid fragment in compounds **1–15**, the two methods agree in allowing this rotation in a wide range of torsion angle values, as well as in predicting a free rotation of the tetralenyl fragments. This is evidence for the high conformational flexibility of arotinoid molecules. This finding may at first seem some-

what surprising because the entire class of aromatic retinoids is often called 'conformationally restricted' retinoids. While this is true when one compares these compounds to retinoic acid where the larger number of single bonds results in large conformational freedom, this term appears to be generally misleading in view of computations presented here. In fact, the remaining two single bonds, 2–11 and 12–13, in the TTNPB molecule (Fig. 3) and its analogs No. 2–15, as well as the only 'bridged' single bond, 12–13, in molecule **19**, still enable free rotation of the left (tetralenyl) aromatic moiety and also rotation of the benzoic acid moiety within the 25–30 to 145–150° range. Thus, the 16 conformers found for each of the compounds **1–14** have low-energy barriers and are interconvertible.

Such conformational behavior may be biologically important because, by allowing the arotinoid molecule a large degree of freedom of ring rotations, it enables the molecule to better fit the receptor surface. A rigid retinoid molecule that prohibits any ring rotation would have much less probability for a favorable orientation at the receptor site. For example, a flexible TTNPB molecule can be argued to have a statistical advantage over the other molecules in the series under examination in fitting the receptor surface. It is generally accepted that the presence of dimethyl substituents of the terminal lipophilic ring at a certain distance from the carboxylic group at the other terminus is a necessary condition for vitamin A derivatives to show anticancer activity [2]. The free rotation of this ring fragment in the TTNPB molecule, which has two dimethyl substituents at positions 5 and 8 (Fig. 3), allows twice as many conformations with the optimum $-(CH_3)_2/-COOH$ distance and torsion angles than in molecules **2–15** having one or no gem-dimethyl group.

The above discussion is not an attempt to explain the biological activity of the examined aromatic retinoids. It is only intended to refer to the possible retinoid molecule–receptor fit, which is a necessary but insufficient condition for an effective drug–receptor interaction. Yet, our conformational analysis provides a basis for better specifying the optimum geometric parameters and for finding the conformationally dependent electronic parameters of these compounds. This information is thus important for building future models of arotinoid anticancer activity that attempt to treat in detail electronic factors and perhaps even solvent involvement.

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