Communications to the Editor

Potent Estrogenic Agonists Bearing Dicarba-*closo*-dodecaborane as a Hydrophobic Pharmacophore

Yasuyuki Endo,* Toru Iijima, Yuko Yamakoshi, Mitsuhiro Yamaguchi, Hiroshi Fukasawa, and Koichi Shudo

Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

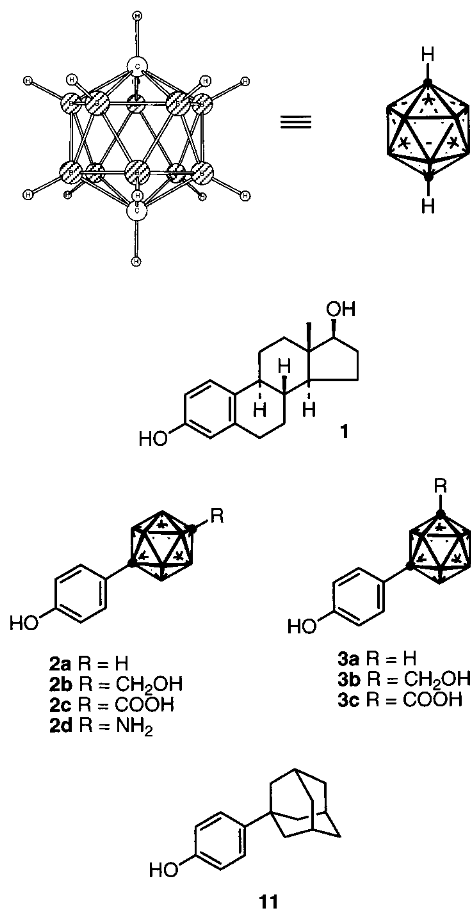
Received February 11, 1999

Introduction. The carboranes (dicarba-*closo*-dodecaboranes)[1] exhibit remarkable thermal stability, are resistant to attack by most types of reagents, and are generally biologically inactive. Their icosahedral geometry, in which the carbon and boron atoms are hexacoordinated, accounts for these unusual properties, which make such molecules and their carbon and boron derivatives uniquely suitable for several specialized applications, including synthesis of polymers for high-temperature use and neutron-shielding purposes.[2] In the field of medical and pharmaceutical sciences, incorporation of large numbers of boron atoms into tumor cells for boron neutron capture therapy (BNCT)[3] has attracted much interest in recent years. For this purpose, various compounds have been synthesized by adding carborane units to nucleic acids,[4] amino acids,[5] etc. In contrast, little attention has been paid to the possible use of carboranes as components of biologically active molecules. The exceptionally hydrophobic character and spherical geometry of carboranes may allow their use as a hydrophobic pharmacophore in biologically active molecules which interact hydrophobically with receptors. Recently, we have reported the first example of design, synthesis, and biological evaluation of retinoids containing a carborane cage as a hydrophobic pharmacophore.[6] In this article, we describe the synthesis and biological evaluation of novel carborane-containing estrogenic agonists which are more potent than 17β-estradiol.

Estrogen (17β-estradiol, **1**) is an important hormone that mediates a wide variety of cellular responses through its binding to a specific nuclear estrogen receptor (ER). The hormone-bound ER forms an active dimer, which functions as a transcription factor that mediates biological response by binding to specific promoter elements of DNA to initiate gene transcription. Compounds that either induce or inhibit cellular estrogen responses have potential value as biochemical tools and candidates for drug development.

Since the discovery of the nonsteroidal estrogen diethylstilbestrol,[7] many stilbene derivatives and triarylethylenes have been synthesized and shown to possess estrogenic activity, and some have been developed for clinical use as estrogen agonists or antagonists.[8] Estrogenic activity was found in a huge range of structural prototypes, including nonsteroidal compounds isolated from plants, such as flavonoids, genistein, and coumesterol.[9] There is currently much debate over the health risks associated with the estrogenic activity of compounds that are either present in the environment or used as chemicals.[10] One of the reasons for the estrogenic activity of such a wide variety of organic chemicals appears to be that ER binding is primarily the result of interaction between the receptor and a phenolic residue. However, high-binding affinity for ER and the appearance of substantial estrogenic activity require a phenolic ring, an appropriate hydrophobic group adjacent to the phenolic ring, and another hydroxyl group located at a suitable position on the molecule.

Chart 1

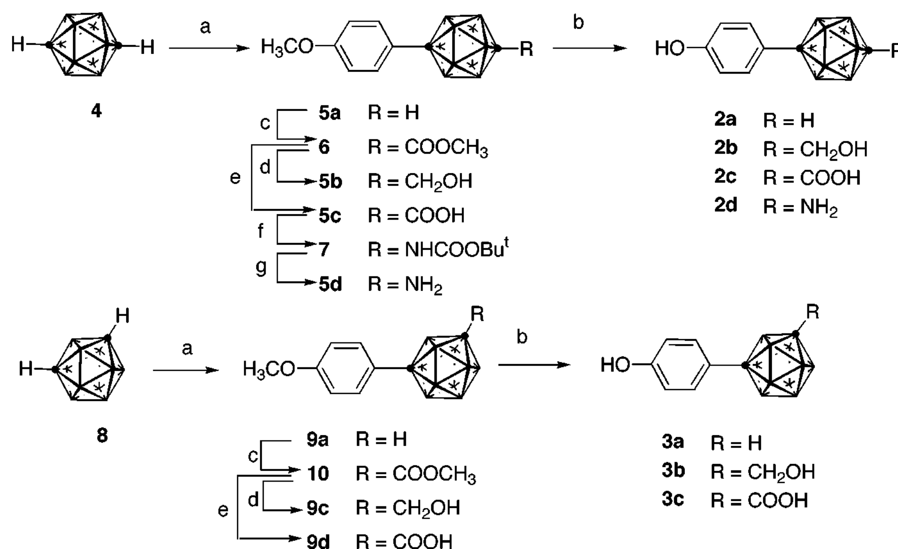


These considerations led us to synthesize and biologically evaluate compounds having carborane as a hydrophobic moiety, with a hydrophilic group on the carborane cluster (**2** and **3**), as shown in Chart 1. In icosahedral cage structures throughout this paper, closed circles (•) represent carbon atoms and other vertexes represent BH units.

Chemistry. The syntheses of the designed molecules are summarized in Scheme 1. Compounds **2a**−**d** were prepared from 1,12-dicarba-*closo*-dodecaborane (**4**). Cou-

10.1021/jm9900725 CCC: \$18.00 © 1999 American Chemical Society
Published on Web 04/17/1999

Scheme 1[a]



[a] (a) (1) *n*-BuLi, CuCl/DME, (2) *p*-iodoanisole/pyridine reflux; (b) BBr_3/CH_2Cl_2; (c) (1) *n*-BuLi/benzene$-Et_2O$, (2) $ClCOOCH_3$; (d) $LiAlH_4$/THF; (e) KOH/H_2O-THF; (f) DPPA, Et_3N, DMAP/*t*-BuOH, reflux; (g) CF_3COOH/CH_2Cl_2.

pling of the *C*-copper(I) derivative of **4**, prepared from the corresponding lithiocarborane, with 4-methoxyiodobenzene in dimethoxyethane in the presence of pyridine gave the mono-C-arylated product **5a** in 60% yield.[11] Demethylation of the methoxy group of **5a** with boron tribromide afforded 1-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**2a**) in 93% yield. Compound **5a** was converted to the *C*-methoxycarbonyl derivative **6** by reaction of the lithiate of **5a** with methyl chloroformate (91%). After reduction of **6** with $LiAlH_4$, demethylation of the methoxyl group gave 1-(hydroxymethyl)-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**2b**) in 98% yield. Hydrolysis of the ester group of **6** followed by demethylation afforded 12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane-1-carboxylic acid (**2c**) in 98% yield. The acid **5c** was converted to the *C*-*tert*-butoxycarbonylamino derivative **7** by means of the modified Curtius rearrangement[12] employing diphenyl phosphorazidate (DPPA), 4-*N,N*-(dimethylamino)pyridine (DMAP), and *tert*-butyl alcohol in 41% yield. Deprotection of the Boc group of **7** followed by demethylation gave 1-amino-12-(4-hydroxyphenyl)-1,12-dicarba-*closo*-dodecaborane (**2d**) in a quantitative yield.

Compounds bearing a meta substituent on the carborane cage, 1-(4-hydroxyphenyl)-1,7-dicarba-*closo*-dodecaborane (**3a**), 1-(hydroxylmethyl)-7-(4-hydroxyphenyl)-1,7-dicarba-*closo*-dodecaborane (**3b**), and 7-(4-hydroxyphenyl)-1,7-dicarba-*closo*-dodecaborane-1-carboxylic acid (**3c**), were prepared from 1,7-dicarba-*closo*-dodecaborane (**8**) via **9a** and/or **10** in the same manner as described for the *p*-carboranyl isomers.

Biology. The estrogenic activities of the synthesized compounds were examined by luciferase reporter gene assay,[13] in which rat ERα-expression plasmid[14] and a reporter plasmid, which contains five copies of estrogen response elements, are transiently transfected into COS-1 cells. 17β-Estradiol at $1 \times 10^{-10}-1 \times 10^{-8}$ M induced the expression of luciferase in a dose-dependent manner.[15] This activation by 17β-estradiol was dependent upon the expression of ER and was completely inhibited by estrogen antagonists (tamoxifen and ICI 164,384). Therefore, the assay system is ER-dependent

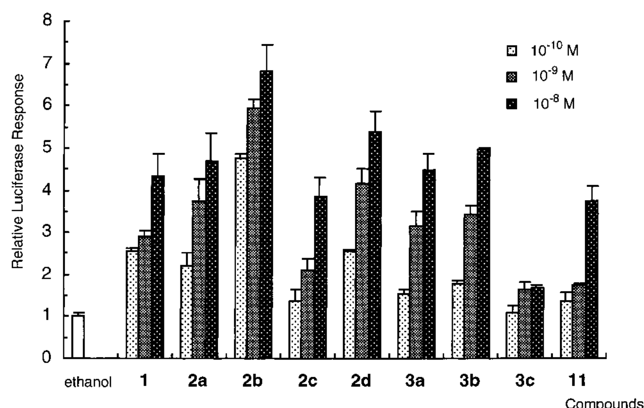


Figure 1. Transcriptional activation by the test compounds. COS-1 cells were transfected with EREx5-pGL-TK and pCI-rERα and incubated with the compounds at the indicated concentrations ($10^{-10}-10^{-8}$ M). Results are shown by means ± SD for triplicate transfections.

and sufficiently sensitive to identify estrogenic compounds and antiestrogens. When cells were treated with antiestrogens, however, the level of expressed luciferase activity was lower than the control level (only ethanol was added to the medium). This suggests that estrogenic activity was not completely excluded from the assay medium or was present intracellularly in the cells used for the assay or that ER was partially activated in a ligand-independent fashion under our assay conditions.

We then examined the estrogenic activity of our carborane-containing molecules (**2** and **3**), and the results are summarized in Figure 1. The compound bearing *p*-carboranyl on the 4-position of phenol (**2a**) exhibited a potent transcriptional activity in the concentration range of $1 \times 10^{-10}-1 \times 10^{-8}$ M; its potency is comparable to that of 17β-estradiol. 4-(1-Adamantyl)-phenol (**11**), in which the substituent resembles the carborane cage in molecular size and shape, also showed moderate activity. 1-Phenyl-1,12-dicarba-*closo*-dodecaborane, which lacks the hydroxyl group, showed completely no activity. The result indicates that the hydroxyl group on the benzene nucleus is essential for the

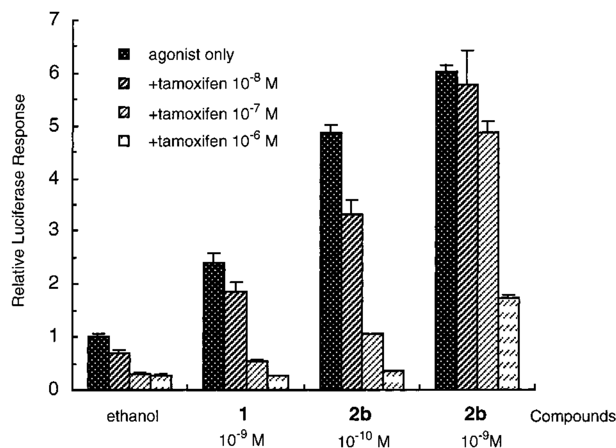


Figure 2. Inhibition by estrogen antagonists. COS-1 cells were transfected with EREx5-pGL-TK and pCI-rERα and incubated with no agonist (ethanol) or with the agonists (10^{-9} or 10^{-10} M) plus the antagonist tamoxifen (10^{-8}–10^{-6} M).

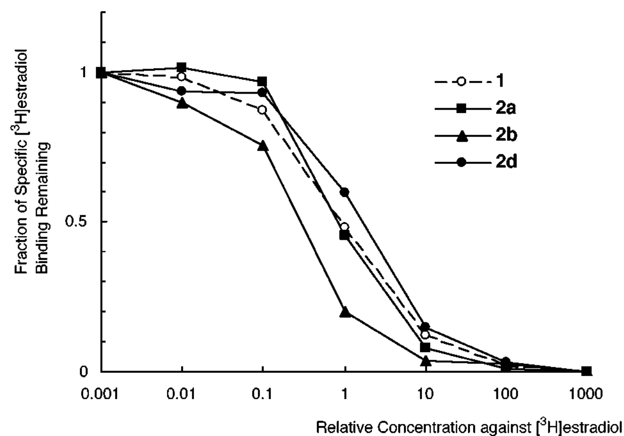


Figure 3. Inhibition of specific [^{3}H]estradiol binding with human recombinant ERα.

appearance of the activity. On the other hand, the activity was increased by the introduction of a hydroxymethyl group or an amino group onto the carbon of the carborane cage. The potency of the most potent compound, **2b**, was at least 10-fold greater than that of 17*β*-estradiol. Tamoxifen inhibited the activity of **2b**, as it did that of 17*β*-estradiol, supporting the idea that the activity of these carborane-containing compounds is mediated by ER (Figure 2). The activity of **2d** seemed to be somewhat stronger than that of 17*β*-estradiol, and **2c** exhibited moderate activity. The activity of the compounds bearing a meta substituent on the carborane cage (**3a**,**b**) was weaker than that of the para-substituted compounds; however, the potency of **3b** is similar to that of 17*β*-estradiol. On the other hand, **3c** was almost inactive and showed no antagonistic activity (data not shown).

In vitro ERα binding assays were performed on the three most active compounds (**2a**,**b**,**d**) and 17*β*-estradiol to confirm that the gene regulatory activity correlated with the binding affinity for the ERα. The assays were done by measurement of inhibition of [6,7-^{3}H]-17*β*-estradiol binding (K_d = 0.4 nM) to human recombinant ERα (PanVera), using the nitrocellurose filter binding assay method. The ERα binding data for these compounds are consistent with the results of the luciferase reporter gene assay (Figure 3). Compounds **2a**,**d** showed strong affinity for ERα; the potency was almost the same as that of 17*β*-estradiol. The most active compound in the luciferase reporter gene assay, **2b**, also showed the highest affinity for the ERα, and its affinity was higher than that of 17*β*-estradiol. The K_i's of **2a**,**b**,**d** for ERα were 0.40, 0.10, and 0.65 nM, respectively.

Discussion. Recent studies on the three-dimensional structure of the complex formed by 17*β*-estradiol and the human estrogen receptor-α ligand binding domain (hERαLBL) have revealed the structural requirements for the appearance of estrogenic activity.[16] 17*β*-Estradiol is oriented in the hERα ligand binding pocket by two types of contacts: hydrogen bonding at both ends and hydrophobic van der Waals contacts along the body of the skeleton. The phenolic 3-hydroxyl group is hydrogen-bonded to the glutamate (Glu-353) of hERαLBD and the 17*β*-hydroxyl group is hydrogen-bonded to the *δ*-nitrogen of His-524.[16] The phenolic hydroxyl group and

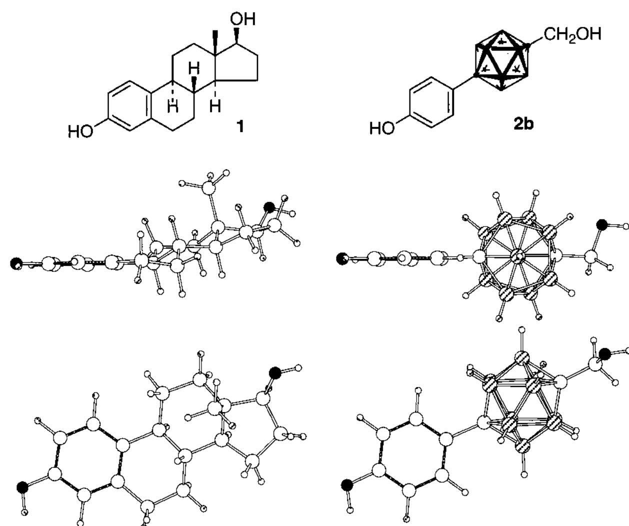


Figure 4. Top: Structures of **1** and **2b**. Middle: Views from the side of the phenolic ring. Bottom: Views from the face of the phenolic ring.

hydroxymethyl group on the carborane cage of **2b** may play similar roles to the phenolic hydroxyl group and 17*β*-hydroxyl group of **1**, respectively, since their relative positions appear to be very similar, as shown in Figure 4. Although the hydrophobic residues implicated in direct binding in the hERαLBD and another steroid receptor (hPRLBD) structures are conserved, a characteristic feature of hERαLBD is the bulky side chain of Leu-387, which seems to recognize the flat aromatic A-ring and the absence of a 19-methyl group of 17*β*-estradiol. However, the hERα ligand binding pocket seems to have some latitude around the C-ring and D-ring of 17*β*-estradiol, as indicated by a three-dimensional quantitative structure–activity relationship study of diethylstilbestrol derivatives using comparable molecular field analysis.[17] The high activity of **2b** suggests that the carborane cage works as a hydrophobic group for binding to the hydrophobic cavity of ER, and the hydrophobic van der Waals contacts along the spherical carborane cage produce a stronger interaction than that in the case of 17*β*-estradiol.[18]

In summary, we have developed novel carborane-containing molecules with potent estrogenic activity. The unique character of biologically active molecules containing a carborane skeleton may give rise to un-

usual membrane transport characteristics and metabolism, compared with conventional active molecules. The superagonistic properties of the carborane-containing compounds raise the possibility that structure–function studies could lead to the development of more selective estrogen agonists and antagonists, which could be useful as therapeutic agents for a wide variety of conditions.

Supporting Information Available: Details of synthesis, spectral data for compounds **2a–d** and **3a–c**, and experimental procedures of biological evaluations. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For a recent review, see: Bregradze V. I. Dicarba-*closo*-dodecaboranes $C_2B_{10}H_{12}$ and their derivatives. *Chem. Rev.* **1992**, *92*, 209–223.
(2) For a recent review, see: Plesek, J. Potential application of boron cluster compounds. *Chem. Rev.* **1992**, *92*, 269–286.
(3) For a recent review, see: Soloway, A. H.; Tjarks, W.; Barnum, B. A.; Rong, F.-G.; Barth, R. F.; Codogni, I. M.; Wilson, J. G. The chemistry of neutron capture therapy. *Chem. Rev.* **1998**, *98*, 1515–1562.
(4) Yamamoto, Y.; Seko, T.; Nakamura, H.; Nemoto, H.; Hojo, H.; Nukai, N.; Hashimoto, Y. Synthesis of carboranes containing nucleoside bases. Unexpectedly high cytostatic and cytocidal toxicity towards cancer cells. *J. Chem. Soc., Chem. Commun.* **1992**, 157–158. Tjarks, W.; Anisuzzaman, A. K. M.; Liu, L.; Soloway, A. H.; Barth, R. F.; Perkins, D. J.; Adams, D. M. Synthesis and in vitro evaluation of boronated uridine and glucose derivatives for boron neutron capture therapy. *J. Med. Chem.* **1992**, *35*, 1628–1633.
(5) Leukart, O.; Caviezel, M.; Eberle, A.; Escher, E.; Tun-Kyi, A.; Schwyzer, R. L- Carboranylalanine, a boron analogue of phenylalanine. *Helv. Chim. Acta* **1976**, *59*, 2184–2187. Wyzlic, I. M.; Soloway, A. H. A general, convenient way to carborane-containing amino acids for boron neutron capture therapy. *Tetrahedron Lett.* **1993**, *33*, 7489–7492. Radel, P. A.; Kahl, A. B.; Enantioselective synthesis of L- and D-carboranylalanine. *J. Org. Chem.* **1996**, *61*, 4582–4588.
(6) Iijima, T.; Endo, Y.; Tsuji, M.; Kawachi, E.; Kagechika, H.; Shudo, K. Dicarba-*closo*-dodecaboranes as a pharmacophore. Retinoidal antagonists and potential agonists. *Chem. Pharm. Bull.* **1999**, *47*, 398–404. Endo, Y.; Iijima, T.; Ohta, K.; Kagechika, H.; Kawachi, E.; Shudo, K. Dicarba-*closo*-dodecaboranes as a Pharmacophore. Nonel Potent Retinoidal Agonists. *Chem. Pharm. Bull.* **1999**, *47*, 585–587.
(7) Dodds, E. C.; Goldberg, L.; Lawson, W.; Robinson, R. Estrogenic activity of alkylated stilbestrol. *Nature* **1938**, *142*, 34.
(8) Ray, S.; Dwivedy, I. Development of estrogen antagonists as pharmaceutical agents. In *Advances in Drug Research*; Testa, B., Meyer, U. A., Eds.; Academic Press: San Diego, 1997; Vol. 29, pp 171–270.
(9) Miksicek, R. J. Commonly occurring plant flavonoids have estrogenic activity. *Mol. Pharmacol.* **1993**, *44*, 37–43.
(10) Stone, R. Environmental estrogens stir debate. *Science* **1994**, *265*, 308–310.
(11) Coult, R.; Fox, M. A.; Gill, W. R.; Herbertson, P. L.; MacBride, J. A. H.; Wade, K. C-Arylation and C-heteroarylation of icosahedral carboranes via their copper(I) derivatives. *J. Organomet. Chem.* **1993**, *462*, 19–29. Fox, M. A.; MacBride, J. A. H.; Peace, R. J.; Wade, K. Transmission of electronic effects by icosahedral carboranes; skeletal carbon-13-chemical shifts and ultraviolet–visible spectra of substituted aryl-*p*-carboranes (1,12-dicarba-*closo*-dodecaboranes). *J. Chem. Soc., Dalton Trans.* **1998**, 401–411.
(12) Kahl, S. B.; Kasar, R. A. Simple, high-yield synthesis of polyhedral carborane amino acids. *J. Am. Chem. Soc.* **1996**, *118*, 1223–1224.
(13) Meyer, T.; Koop, R.; von Angerer, E.; Schonenberger, H.; Holler, E. A rapid luciferase transfection assay for transcription activation effects and stability control of estrogenic drugs in cell cultures. *J. Cancer Res. Clin. Oncol.* **1994**, *120*, 359–364.
(14) Koike, S.; Sakai, M.; Muramatsu, M. Molecular cloning and characterization of rat estrogen cDNA. *Nucleic Acids Res.* **1987**, *15*, 2499–2513.
(15) The luciferase response was almost saturated above 10^{-8} M when 17β-estradiol was used in this assay.
(16) Brzozowski, A. M.; Pike, A. C. W.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engstrom, O.; Ohman, L.; Greene, G. L.; Gustafsson, J.; Carlquist, M. *Nature* **1997**, *389*, 753–758. Tanenbaum, D. M.; Wang, Y.; Williams, S. P.; Sigler, P. B. Crystallographic comparison of the estrogen and progesterone receptor's ligand binding domains. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 5998–6003.
(17) Sadler, B. R.; Cho, S. J.; Ishaq, K. S.; Chae, K.; Korach, K. S. Three-dimensional quantitative structure–activity relationship study of nonsteroidal estrogen receptor ligands using the comparable molecular field analysis/cross-validated r^2-guided region selection approach. *J. Med. Chem.* **1998**, *41*, 2261–2267.
(18) The relatively high activity of 4-(1-adamantyl)phenol (**11**) also suggests that the hydrophobic contact along the spherical shape produces a stronger interaction than that in the case of other alkylphenols.

JM9900725