# Molecular Structure and Activity Toward DNA of Baicalein, a Flavone Constituent of the Asian Herbal Medicine "Sho-saiko-to"

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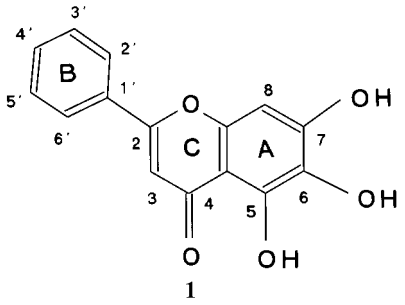
Baicalein (5,6,7-trihydroxyflavone, **1**) is of interest because of its broad spectrum of biological activity. It is a constituent of the east Asian herbal remedy, "Sho-saiko-to". The 3D structure of **1** was determined using X-ray diffraction. The compound exists in an almost planar conformation with a C-2—C-1′ bond distance of 1.476(5) Å. Hydrogen-bonding interactions predominate in the crystal structure. The position of the three hydroxyl groups maximizes intramolecular hydrogen bonding, and each of the hydroxyl hydrogen atoms is a donor in a three-center hydrogen bond. The carbonyl oxygen, O-4, is an acceptor in an intramolecular hydrogen bond (with OH-5). Two molecules of **1** exist as hydrogen-bonded dimers related by inversion center ($-x + 1, -y, -z + 1$). O-4 is also an acceptor in an intermolecular hydrogen bond with OH-6. The planarity of the flavone framework is dependent on structural and/or electronic forces that stabilize the negative charge on the exocyclic oxygen atom, O-4. Compound **1**, therefore, is planar in any situation where forces can stabilize the negative charge on O-4. Consistent with this, UV absorbance studies performed on **1**−DNA complexes with varying concentrations of **1** strongly suggest intercalation of **1** within the double helix, followed by possible interstrand cross-links.

Flavones are a class of naturally occurring phenolic plant compounds that show biological and pharmacological activity coupled with low toxicity.[1] These compounds are widely distributed in the plant kingdom and ingested daily by humans. Therefore, their use as potential therapeutic compounds against a variety of diseases is of interest.[2] A variety of biological activities have been attributed to baicalein (5,6,7-trihydroxyflavone, **1**). It is found in many plants[3] and is a major component of the widely used east Asian herbal medicine, "Sho-saiko-to". This remedy is a combination of seven herbs that include *Scutellaria baicalensis* Georgi (Labiatae) root, *Bupleurum falcatum* L. (Umbelliferae) root, *Pinellia ternata* Breitenbauch (Araceae) tuber, *Zizyphus vulgaris* Lam. (Rhamnaceae) fruit, *Panax ginseng* C. A. Meyer (Araliaceae) root, *Glycyrrhiza glabra* L. (Leguminosae) root, and *Zingiber officinale* Roscoe (Zingiberaceae) rhizome,[4] and is effectively used to treat chronic hepatitis in Japan.[5]




**1**

As are other flavonoids, baicalein is a strong antioxidant and shows free-radical scavenging ability.[1,6,7] Antioxidants also prevent cellular components such as lipids from becoming oxidized. Oxidation of lipids circulating in the blood is the initial step in atherosclerosis, as well as being associated with the hepatic fibrosis experienced in hepatitis. Because **1** inhibits lipid peroxidation in rat liver mitochondria,[5] this may explain why "Sho-saiko-to" is used effectively to treat chronic hepatitis in Japan. Superoxide radical circulation in tissues has been implicated in several pathological processes such as atherosclerosis, inflammation, and cancer, as well as aging.[8] Compound **1** has been shown to prevent human dermal fibroblast cell damage induced by reactive oxygen species,[9] as well as liposome membrane damage caused by UV exposure (and subsequent free-radical appearance).[10] As an antioxidant, **1** is also an inhibitor of xanthine oxidase, which plays a major role in gout. [11]

In addition, baicalein shows antiinflammatory activity, probably because it selectively inhibits 12-lipoxygenase (one of a family of dioxygenases involved in arachidonic acid oxidation).[12] As for its activity toward the cyclooxygenases, the other enzymes involved in arachidonic acid oxygenation to form prostaglandins, there are conflicting results regarding the inhibitory role of **1**.[13,14]

Baicalein has been shown to be a strong and highly specific inhibitor of two reverse transcriptases from human immunodeficiency virus (HIV) and Rauscher murine leukemia virus (RLV).[15,16] Compound **1** is active against HIV-1 integrase.[17] It induces mammalian topoisomerase II-dependent DNA cleavage in vitro[18] and so causes cytotoxic lesions in cells.

The efficacy with which baicalein inhibits many biological systems implicated in human disease prompted us to study its molecular structure. Because the structure affects its activity, we present here the 3D structure of **1**, along with some studies on its interactions with DNA.

## Results and Discussion

Crystal data for baicalein (**1**) are given in Table 1, while final fractional atomic coordinates are listed in Table S1,

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**Table 1.** Crystal Data for Baicalein (**1**)

| | |
|---|---|
| elemental formula | $C_{15}H_{10}O_5$ |
| $M_r$ | 270.24 |
| $a$ | 7.907(4) Å |
| $b$ | 13.367(5) Å |
| $c$ | 12.911(6) Å |
| $\beta$ | 117.26(2)° |
| $V$ | 1213.1(6) Å$^3$ |
| $z$ | 4 |
| cryst syst | monoclinic |
| space group | $P2_1/n$ (#14) |
| temperature | 25 °C |
| $D_c$ | 1.48 g cm$^{-3}$ |
| $F$(000) | 560 |
| $\mu$(Cu K$\alpha$) | 9.603 cm$^{-1}$ |
| $\lambda$ | 1.5418 Å |
| (sin $\theta$)/$\lambda_{max}$ | 0.59 ($2\theta_{max}$ =130°) |
| cryst size | 0.40 × 0.20 × 0.20 mm |
| min/max transmn | 0.89/1.00 |
| cryst growth method | evaporation |
| solvent syst | 3:1 methanol−water |
| data collection apparatus | Crystal Logic SYNTEX $P2_1$ diffractometer |
| no. of reflns to determine cell | 22 (chosen between 5−20° in 2$\theta$) |
| total no. of reflns | 2324 |
| range $h$, $k$, $l$ | 0→9, 0→15, −15→13 |
| reflections with $I > 3\sigma(I)$ | 1656 |
| $R$ | 0.0584 |
| $R_w$ | 0.0839 |
| abs corr ($\varphi$-scan) | none |
| decay correction (decay = 2.6%) | none |

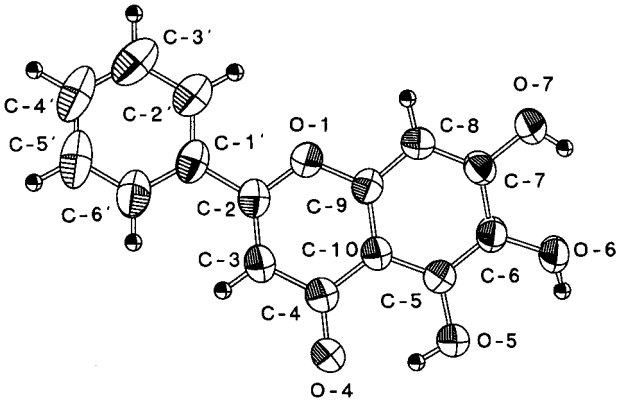


**Figure 1.** ORTEP30 diagram of **1** showing nomenclature and ellipsoids at 50% probability.

Supporting Information. Intramolecular bond distances and angles are as expected (Table S2, Supporting Information) and comparable to similar compounds, such as quercetin, another biologically active flavonoid.[19] The C-2—C-1′ bond connecting rings C and B is usually described as a single bond having free rotation; therefore, the two rings do not have to be coplanar. However, in **1**, this bond distance is 1.476(5) Å, and clearly has double-bond character, implying a greater contribution from a resonance structure in which there is a C-2—C-1′ double bond and a planar conformation. As seen in Figure 1, **1** exists in an almost planar conformation. The orientation of the phenyl ring to the rest of the flavonoid structure also can be measured by the torsion angle about the bond C-2—C-1′. The O-1—C-2—C-1′—C-2′ torsion angle is 8.2(5)°.

Analysis of the factors that affect the planarity of the flavone framework has been discussed in an earlier work by our group.[19] Conformational energy analysis of the flavonoid skeleton reveals that the lowest potential energy corresponds to a nonplanar conformation in which the torsion angle is 22.8° about the C-2—C-1′ bond.[20] This intermediate torsion angle represents a balance between the attractive interaction from a planar structure that provides the framework for a more extensive mesomeric effect and the repulsive steric interactions that would result from the two ortho hydrogen atoms in the configuration in which the rings are coplanar. The difference in energy between the lowest energy (22.8°) conformation and the planar one is 0.7 kcal/mol.

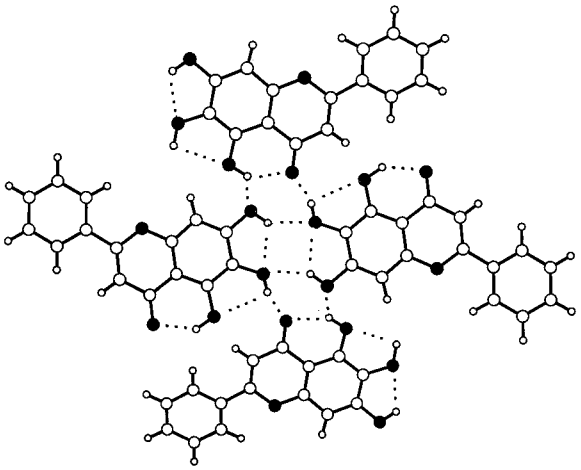

**Figure 2.** Hydrogen-bonding network as described in Table S3 in the **1** crystal structure. View is roughly down the $x$ axis, and the oxygen atoms are shaded.

The exocyclic oxygen, O-4, is crucial to establishing the planarity of the flavone structure. Stabilization of the partial negative charge on O-4 via hydrogen bonding interactions increases the contribution of the mesomeric effect. Flavones can therefore be expected to be planar in any situation where there are forces that can stabilize the negative charge on O-4. These forces include metal-ion coordination through O-4, interaction with amino acids that contain hydrogen-bond donors, and intercalation stacking interactions with DNA. Any of these factors could provide sufficient energy to overcome the small energy barrier so that the molecule can adopt the planar conformation. This observation is confirmed by the structure determination of **1**, in as much as since O-4 is involved in two strong hydrogen bonds, one an intramolecular hydrogen bond with neighboring OH-5 at 2.625(4) Å and the other an intermolecular hydrogen bond with OH-6 at 2.640(4) Å.

The complete network of hydrogen bond parameters is provided in Table S3 (Supporting Information) and shown in Figure 2. There are intramolecular hydrogen-bonding interactions involving the three hydroxyl groups (O-5, O-6, and O-7), where each of the hydroxyl hydrogen atoms acts as a donor in a three-center hydrogen-bonding situation. The crystal structure shows intermolecular hydrogen-bonding interactions as well. Two molecules of **1** exist as hydrogen-bonded dimers related by inversion center (−x + 1, −y, −z + 1). Apart from these hydrogen bonds, the crystal structure is held together by the stacking interactions of the almost planar hydrogen-bonded dimer molecules. The stacking of the molecules is roughly perpendicular to the (101) diffraction plane (Figure 3).

Baicalein (**1**) has a strong absorbance peak in the UV region at 272 nm and a minor peak at 320 nm. Complexes of **1** with mononucleotides were found to exhibit composite spectra with $\lambda_{max}$ at 267−269 nm (Figure 4). Quantitative analysis of these spectra indicated a possible interaction between the purine nucleotides and **1**. However, the pyrimidine nucleotide complexes exhibited absorbances and $\lambda_{max}$ as predicted by quantitative calculations for mixtures of noninteractive components.

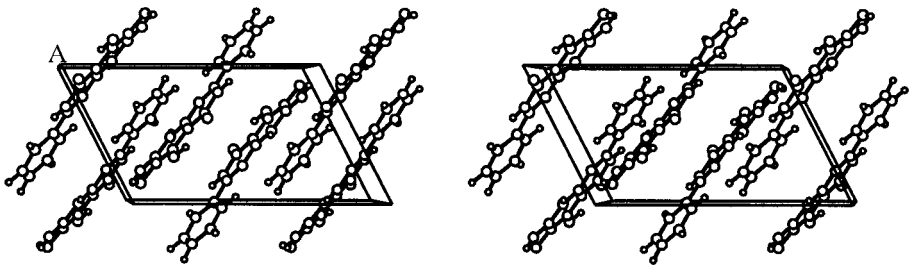


**Figure 3.** Stereoview of unit cell (down *y* axis) showing stacking of the **1** molecules roughly perpendicular to the (*101*) diffraction plane.

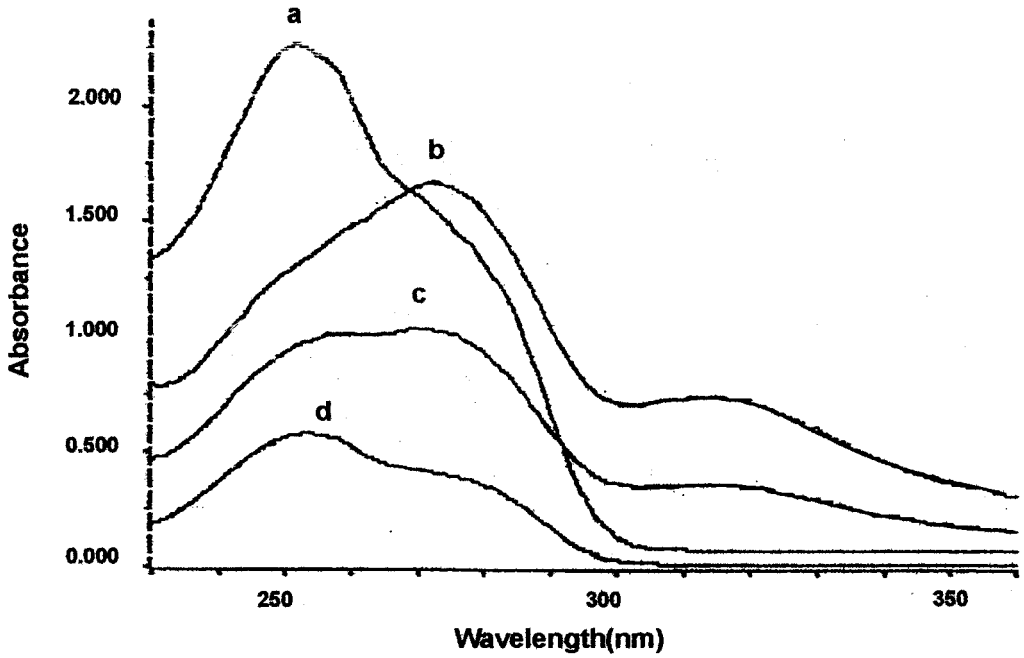


**Figure 4.** UV absorbance spectra of baicalein (**1**) and its complexes with GMP: (a) **1**, (b) 1:1 complex of **1**−GMP, (c) 0.5:1 complex of **1**−GMP, (d) GMP.

Complexes with **1**-to-DNA ratios of 0.5:1, 1:1, and 2:1 exhibited more than the calculated increase in absorbance at room temperature (Figure 5). Also, it was seen from the melting curves of DNA and its complexes with **1**, that the hyperchromicity due to denaturation was quite low (Figure 6a,b). A significant effect seen at 1:1 and 2:1 ratios of **1** to DNA is the post-melting decrease in absorbance, which strongly suggests that either the helix-coil transition in the **1**−DNA complex is complete at room temperature or is prevented completely due to the two strands being held together by some sort of interaction. We are inclined to eliminate the former conclusion by comparing agarose gel electrophoretic patterns of native DNA, denatured DNA, and the **1**−DNA complexes. Native DNA and its complexes with **1** exhibited sharp bands with similar relative mobilities. Our data combined with the demonstration of planarity and hydrogen bonding capabilities from the X-ray crystallographic data, lead us to hypothesize the following sequence of events (shown in Figure 7) taking place upon interaction of **1** with DNA. The initial intercalation of **1** into one strand of DNA possibly happens with localized unwinding and base destacking, which results in the hyperchromic effect observed in DNA while in the presence of **1** at ambient temperatures. Eventual interstrand covalent cross-linking of **1** between purine residues of one strand to the other is facilitated at higher temperatures, resulting in the formation of a condensed double-stranded DNA structure as shown in Figure 7. This is supported by the hypochromic effect observed at higher temperatures and higher ratios of **1** to DNA. Our data (unpublished results) from the restriction reactions of equimolar **1**−DNA complexes with selected restriction enzymes lend further support to the interstrand cross-linking hypothesis presented here.

Examination of the molecular structure of baicalein (**1**) has revealed at least two ways in which enzyme activity could be reduced or eliminated: (a) Many enzymes have or require metal ions for activity. For example, HIV-1 integrase requires the metal ions ($Mg^{2+}$ or $Mn^{2+}$) for optimal activity.[21,22] Previous studies have implied that metal chelators, for example, **1**, could sequester these metal ions and coordinate the metal through the exocyclic O-4 and the adjacent hydroxyl group.[17] (b) Baicalein (**1**) could act as a template for attractive hydrogen-bonding interactions with the amino acids involved in protein−metal ion coordination and so interfere with metal ion−enzyme coordination. Again, in HIV-1 integrase, there are H−H−C−C residues in the *N*-terminal domain that normally bind $Zn^{2+}$; **1** could bind with these amino acids. It could also bind to the COOH side chains of aspartic acid and glutamic acid in the catalytic core of HIV-1 integrase that normally interact with $Mn^{2+}$.

The inhibitory effect of **1** on HIV integrase can be correlated to the intercalative binding of **1** to DNA. The conformation of the **1**−DNA complex may prevent it from fitting into the DNA binding C-terminal domain of the enzyme. Or, the complex may act as a competitive analogue of native DNA by binding in its place on HIV integrase, thus exhibiting its inhibitory effect. Similar conformational alterations to DNA have been observed through spectro-

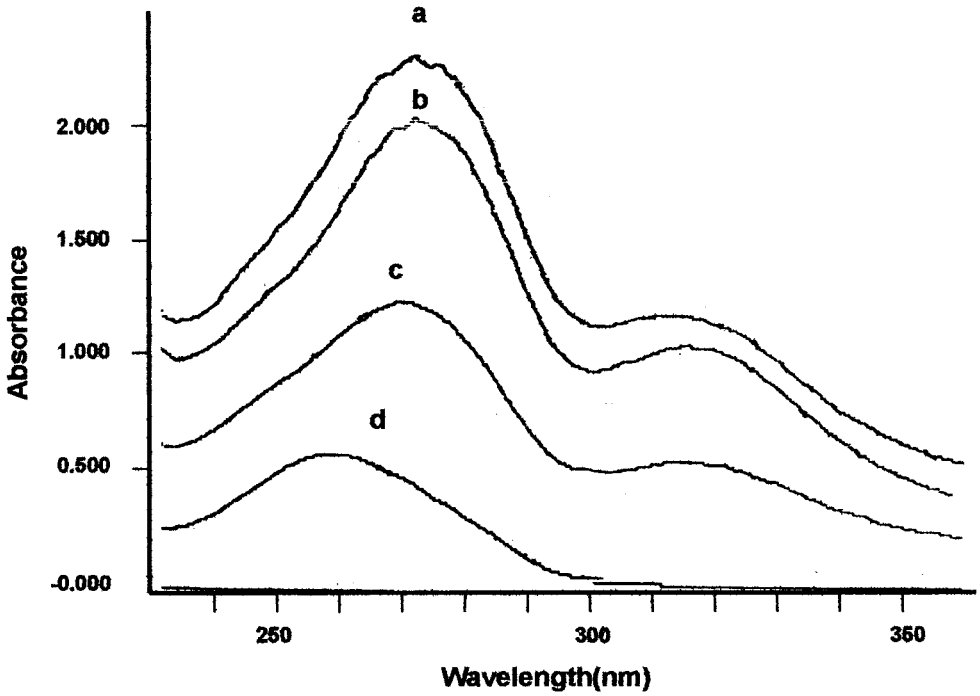


**Figure 5.** UV absorbance spectra of baicalein (**1**) and its complexes with DNA: (a) **1**, (b) 1:1 complex of **1**−DNA, (c) 0.5:1 complex of **1**−DNA, (d) native calf thymus DNA.

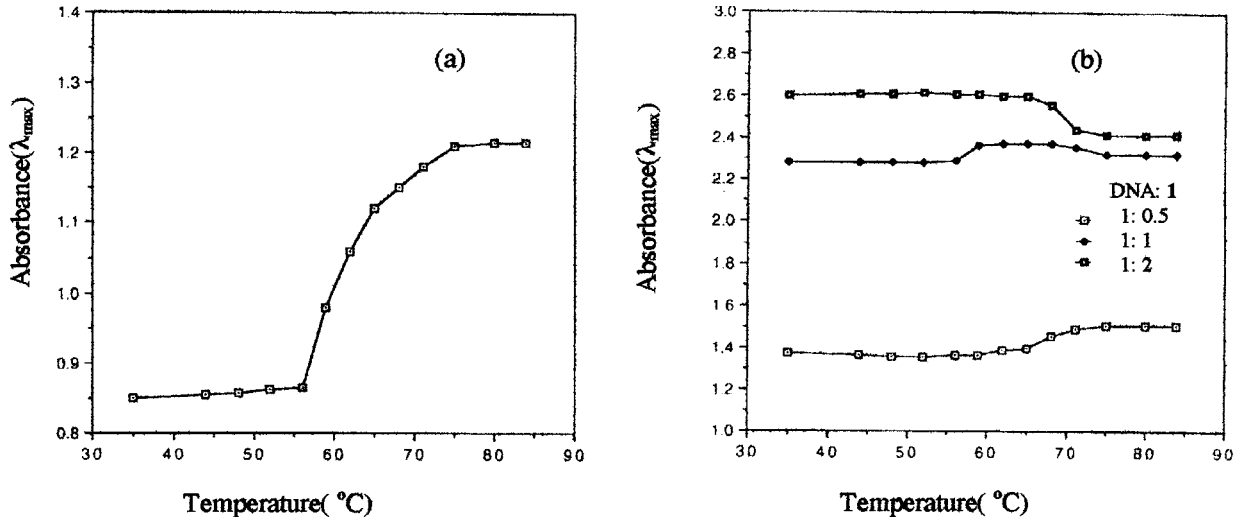


**Figure 6.** Melting curves of (a) calf thymus DNA and (b) its complexes with baicalein (**1**).

scopic evidence of a charge-transfer complex formation when the flavonoid quercetin binds to DNA.[23]

The phenolic groups of flavonoids are crucial to their activity, as flavonoids lacking them are inactive. Structure−activity studies on the location of hydroxyl groups on the flavone skeleton have been carried out to determine the best activity toward flavonoid inhibition of xanthine oxidase and superoxide scavenging activity.[14] Conclusions reached were that a double bond between C-2 and C-3 (to ensure coplanarity of the phenyl ring) and hydroxyl groups at C-5 and C-7 are essential features for inhibition of xanthine oxidase, while hydroxyl groups at C-3 and C-3′ are essential for superoxide scavenging ability.[11] In light of the structure analysis regarding the flavone skeleton described above, it can be seen that the coplanarity of the phenyl ring (and subsequent planarity of the flavone ring) is determined by exocyclic oxygen, O-4, interactions.

Fesen et al. have described the flavone structural features needed for optimal HIV-1 integrase activity: at least three hydroxyl groups are needed, with three adjacent hydroxyls giving the best results.[17] Austin et al. have described structure−activity studies on flavonoid inhibition of DNA binding to topoisomerase II and reached similar conclusions.[18]

Flavone inhibition of cytochrome P450 enzymes has been known for a long time, and many studies have been published to determine the ideal structure of these compounds for optimal activity as P450 inhibitors.[19,20,24] Flavones (and other inhibitors) that are primarily flat, such as quercetin, inhibit P450 enzymes responsible for the carcinogenic oxidation of polycyclic aromatic hydrocarbons. On the other hand, nonplanar inhibitors act on other P450 enzymes, such as those involved in steroid metabolism or aromatase, implicated in breast cancer. Because baicalein (**1**) is planar, it is not surprising that, although some flavones inhibit human aromatase, **1** does not.[25]

## Experimental Section

**Chemicals.** For crystallographic studies, **1** was obtained from Aldrich Chemical Co. For UV absorption studies, calf

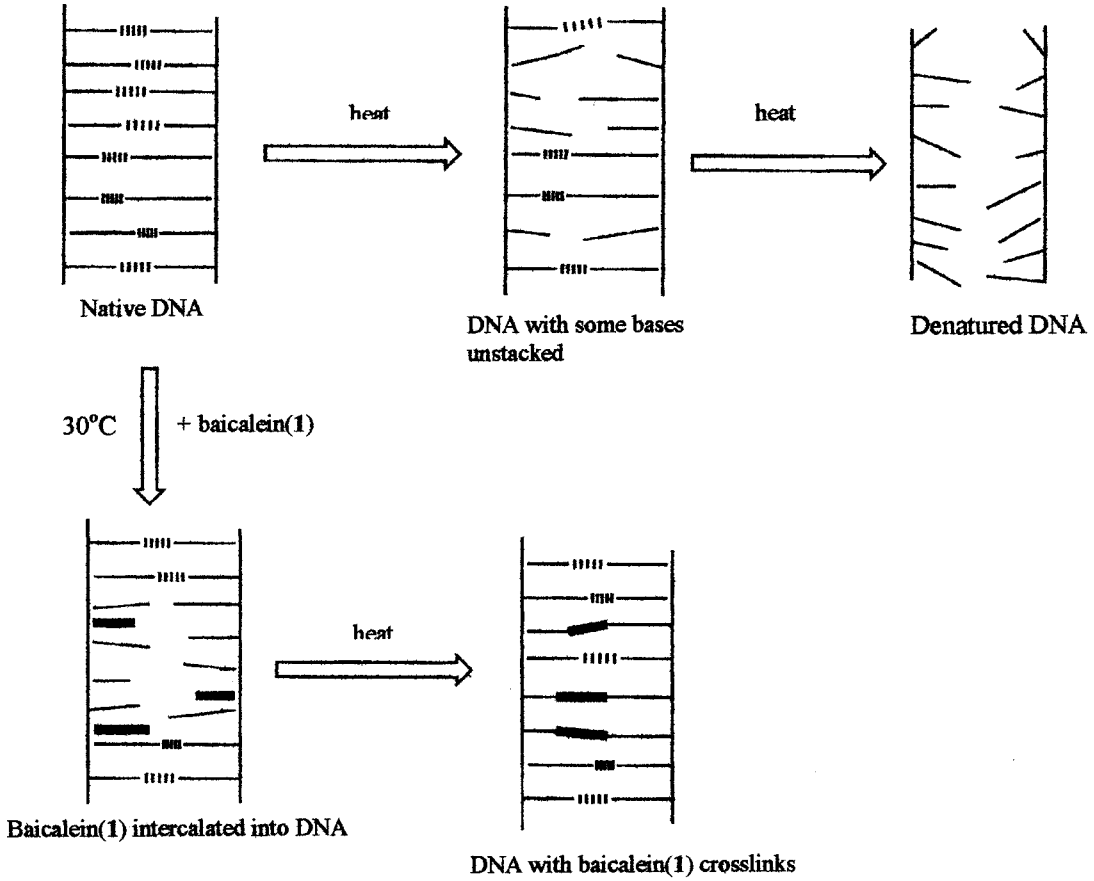


**Figure 7.** Possible model for the interaction of baicalein (**1**) with DNA.

thymus DNA, **1**, and the mononucleotides guanine monophosphate, adenosine monophosphate, cytosine monophosphate, and thymidine monophosphate were purchased from Sigma Chemical Co.

**X-ray Crystallographic Studies.** Suitable crystals formed from an aqueous methanol solution after a few days. Experimental and crystal data are given in Table 1. Intensity data were collected using a Crystal Logic modified Syntex $P2_1$ diffractometer. The structure was solved using direct methods (SHELX-86),[26] and all the heavy atoms were revealed immediately. Isotropic, followed by anisotropic refinement, was performed on the atomic parameters. At this point, the positions of all the hydrogen atoms were found in a Fourier difference synthesis. Inclusion of the hydrogen atom coordinates and allowing them to refine riding on their parent atoms produced the final value of $R = 0.0584$ and $R_w = 0.0839$. The quantity minimized during refinement was $\Sigma w(||F_o| - |F_c||)^2$ with the weighting scheme, $w = 1/(a + F_o + cF_o^2)$, where $a$ and $c$ are on the order $2F_o$(min) and $2F_o$(max), respectively.

Computer programs used were from the CAOS system,[27] and the atomic scattering factors are from a compilation of published values.[28] Drawings were done using the TEXSAN system.[29] Final fractional atomic coordinates for all the atoms are available through Supporting Information and through the CCDC.[31]

**Ultraviolet Absorption Studies.** All solutions were made in Tris-EDTA buffer (pH 7.8) to a final working concentration of 0.2 micromoles/ml. (Note: DNA concentrations in the present studies are expressed as DNA−phosphorus concentrations.) The **1**−DNA and **1**−mononucleotide complexes were prepared by mixing appropriate volumes of the two components to give molar ratios of 0.5:1, 1:1, and 2:1 and incubating at 25 °C for 30 min before obtaining spectra. No spectral changes were observed in any of the solutions on storing for about 48 h in cold. All UV spectra were obtained using an HP8452 UV−vis spectrophotometer equipped with "Advanced Quant" software and jacketed cuvette holders. Temperature control was achieved through circulating an ethylene glycol−water mixture from an Isotemp circulating water bath through the cuvette holders.

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**Supporting Information Available:** Final fractional atomic coordinates and isotropic displacement parameters,bond lengths (Å) and bond angles for **1**, hydrogen bond parameters, anisotropic displacement parameters for non-hydrogen atoms, and table of $F_o$ vs $F_c$ for *hkl* data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(31) Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 (0) 1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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