A Stereoselective Total Synthesis of Estrone by an Intramolecular Cycloaddition Reaction of Olefinic *o*-Quinodimethane[1]

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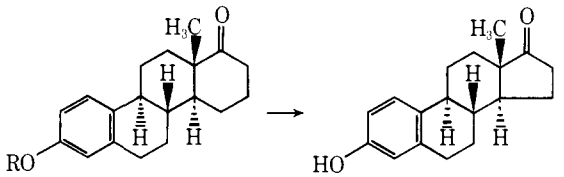
Abstract: A stereo- and regioselective synthesis of *O*-methyl-*D*-homoestrone (**1**) was achieved by an intramolecular cycloaddition of the *o*-quinodimethane (**39**) derived from thermolysis of the benzocyclobutene derivative (**38**), which was obtained by a condensation of β-(4-methoxybenzocyclobutenyl)ethyl iodide (**17**) with 6-*n*-butylthiomethylene-2-methyl-3-vinylcyclohexanone (**34**) followed by removal of the *n*-butylthiomethylene group of the resulting product (**35**).

For more than 30 years, the synthesis of estrone (**3**) has held special interest for organic chemists,[2] partly because of the follicular hormone activity in estrone itself, and partly because estrone is an important precursor in the production of 19-norsteroid[3] which has been used as an oral contraceptive, and many types of approaches have been reported toward this female sex hormone.[2] In the last decade, attention has focused on developing asymmetric syntheses of estrone and related compounds.[4] Recently, Johnson reported a highly efficient stereospecific synthesis of estrone via a cationic olefinic cyclization,[5] and Danishefsky described a novel approach to estrone through *D*-homoestrone based on a bisannelation sequence.[6]

In connection with our interest[7-10] in the synthetic application of the cycloaddition reaction and electrocyclic reaction[11] starting from *o*-quinodimethanes based on benzocyclobutenes,[12-14] we investigated a new and simple total synthesis of estrone (**3**) and *D*-homoestrone (**2**), which has been correlated with estrone,[15-17] via the intramolecular cycloaddition reaction of the olefinic *o*-quinodimethane (**39**) generated from the benzocyclobutene (**38**) by electrocyclic ring opening. This paper reports a synthesis of *D*-homoestrone (**2**), which constitutes a formal total synthesis of estrone (**3**), by the method that provides a general synthetic sequence of A-aromatized steroids having given substituent(s) at given position(s).

Scheme I



1, R = CH_3
2, R = H

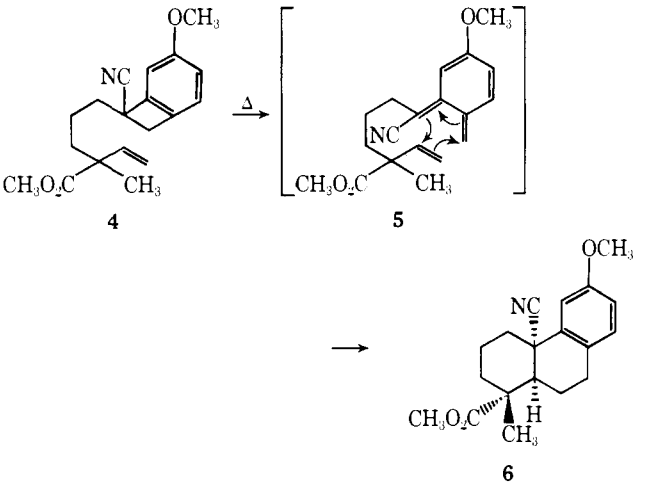
3

Our synthetic principle is that benzocyclobutenes such as **4** readily undergo thermal rearrangement to *o*-quinodimethanes **5**, which can further participate in intramolecular cycloaddition to give the hydrophenanthrene derivatives **6**.[8] Keeping this in mind we designed a novel synthesis of estrone (**3**) and *D*-homoestrones (**1, 2, 32**) from the benzocyclobutenes (**23, 29,** and **38**) via *o*-quinodimethanes (**24, 30,** and **39**).

Results and Discussion

Synthetic Approach to Estrone (3). The preparation of the requisite benzocyclobutene derivative **22** was straightforward and involved the convergent synthesis illustrated in the following schemes. Hydrolysis[18] of 1-cyano-4-methoxybenzocyclobutene (**7**)[10] with excess potassium hydroxide in ethanol,

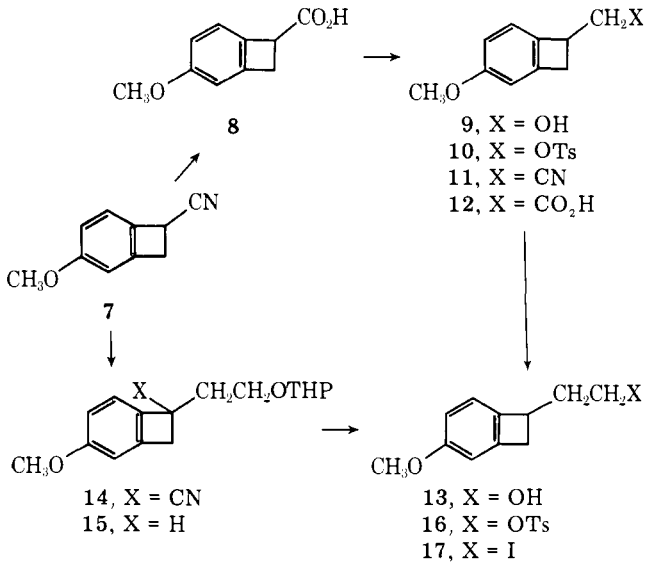
Scheme II



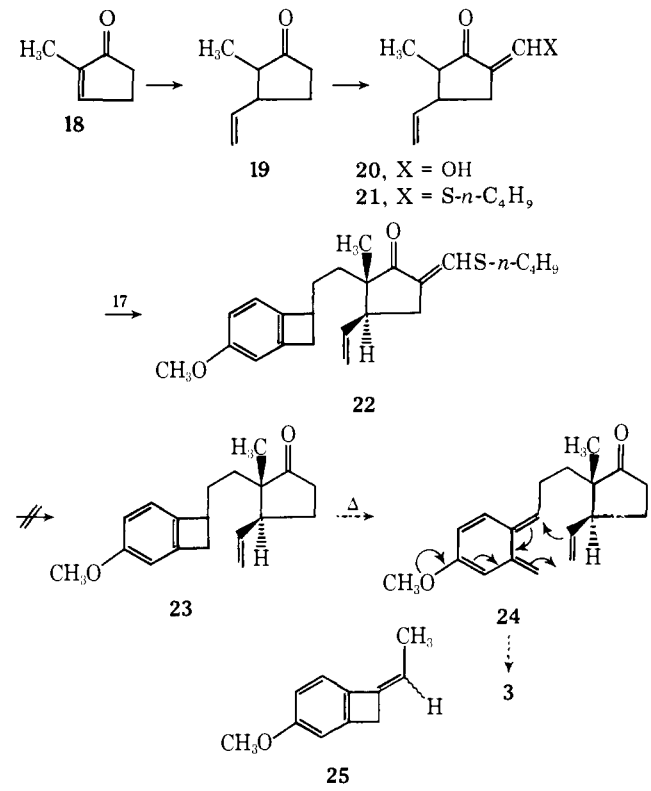
initially at room temperature followed by refluxing, gave the carboxylic acid **8** in 80.4% yield. This carboxylic acid was reduced with lithium aluminum hydride in tetrahydrofuran at room temperature, followed by tosylation of the resulting alcohol **9** with *p*-toluenesulfonyl chloride in pyridine at room temperature to afford in 76% overall yield the tosylate **10**. Cyanation[19] of the tosylate **10** was carried out with sodium cyanide in dimethyl sulfoxide at room temperature and the nitrile **11** thus obtained was treated with ethanolic potassium hydroxide[18] as above to give 1-carboxymethyl-4-methoxybenzocyclobutene (**12**) in 83% overall yield from the tosylate **10**. Lithium aluminum hydride reduction of the acetic acid derivative **12** at room temperature gave quantitatively the corresponding ethyl alcohol derivative **13,** which is also prepared easily from the starting nitrile **7** by the following method. Thus, the tetrahydropyranyl ether[20] derived from ethylene bromohydrin was condensed with the 1-cyanobenzocyclobutene **7** in the presence of sodium amide in liquid ammonia, affording the ethylated 1-cyanobenzocyclobutene **14** in 91% yield. Reductive decyanation[21] of this product **14** using sodium in liquid ammonia, followed by a cleavage of the tetrahydropyranyl group of the resulting compound **15** with hydrochloric acid in methanol, gave the alcohol **13** in 79% overall yield from **14.** Finally, the alcohol **13** was treated with *p*-toluenesulfonyl chloride in pyridine at room temperature to furnish the tosylate **16** in 44% yield, which was converted into 2-(4-methoxybenzocyclobutenyl)ethyl iodide (**17**) by treatment with sodium iodide in boiling acetone in 92% yield.

Firstly, we examined a direct synthesis of the 2-benzocyclobutenylethyl-2-methyl-3-vinylcyclopentanone (**23**) by trapping[22] the regioselectively generated enolate, derived from

Scheme III



Scheme IV



1,4-addition of vinylmagnesium bromide catalyzed by cuprous iodide to **18,** with the iodide **17,** but this trial was not successful. However, the cyclopentane part of **23** was constructed as follows: an introduction of the vinyl group at the β position in 2-methyl-2-cyclopenten-1-one (**18**)[23] with vinylmagnesium bromide in the presence of cuprous iodide in tetrahydrofuran at −78 °C by 1,4-addition reaction[24] furnished in 56.6% yield the 3-vinylcyclopentanone **19**.

In order to introduce regioselectively the benzocyclobutenylethyl iodide **17** to the C_2 position of 2-methylcyclopentanone **19,** the C_5 position of **19** was blocked by the *n*-butylthiomethylene group. Following the procedure of Ireland and Marshall,[25] the cyclopentanone **19** was allowed to react with an excess of ethyl formate in the presence of sodium hydride in benzene at room temperature and the crude 5-hydroxymethylenecyclopentanone **20** was converted into 5-*n*-butylthiomethylenecyclopentanone **21** in 62.5% overall yield by treatment with *n*-butyl mercaptan and *p*-toluenesulfonic acid in boiling benzene with azeotropic removal of water.

With the two building blocks **17** and **21** for a preparation of **23** in hand, condensation of both components was examined. Initial experiments were carried out in the presence of sodium amide as a condensation reagent in liquid ammonia, but in this reaction the main product was a mixture of (*E*)- and (*Z*)-1-ethylidenebenzocyclobutene **25** [*m/e* 160 (M^+)], which showed an olefinic methyl group at 1.76 and 1.86 ppm as a doublet (J = 9.5 Hz) and an olefinic proton at 4.96–5.6 ppm as multiplet in the NMR spectrum. The successful condensation of two compounds **17** and **21** was accomplished by conversion of the latter to its potassium salt with potassium *tert*-butoxide in *tert*-butyl alcohol, followed by addition of the iodide **17,** and subsequent stirring of the mixture at 20 °C for 16 h and then 80 °C for 16 h to form the 2,2-disubstituted cyclopentanone **22** in 25.5% yield after silica gel chromatography. However, upon hydrolysis of this condensation product in order to remove the protecting *n*-butylthiomethylene group under various basic or acidic conditions, undesired reactions occurred to give undefined products, but not the expected compound **23**.

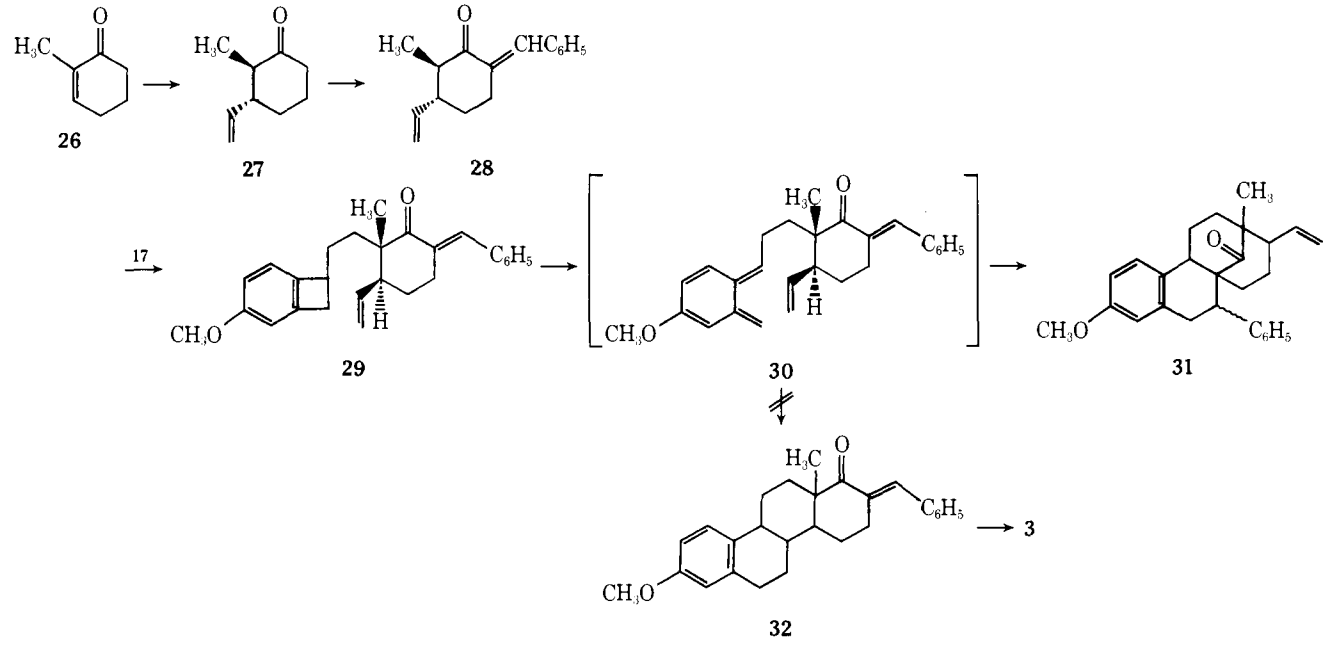
Synthesis of *D*-Homoestrone. Since formation of the benzocyclobutene derivative **23** having the cyclopentanone system could not be achieved, attention was directed at the intramolecular cycloaddition of the benzocyclobutene **29** and **38** having the cyclohexanone ring, which would be more stable than the cyclopentanone system to acids and bases.

Firstly, 6-benzal-2-[2-(4-methoxybenzocyclobutenyl)-ethyl]-2-methyl-3-vinylcyclohexan-1-one (**29**) was prepared by a three-step procedure as shown in a following scheme; 1,4-addition[24] of vinylmagnesium bromide to 2-methyl-2-cyclohexen-1-one (**26**)[26] in the presence of cuprous iodide in tetrahydrofuran at −78 °C gave the 3-vinylcyclohexanone **27** in 66% yield, which was converted into the 6-benzylidene derivative **28** by a treatment with benzaldehyde in the presence of sodium hydroxide in boiling aqueous ethanol.[27] This compound **28** was condensed with benzocyclobutenylethyl iodide **17** in the presence of potassium *tert*-butoxide in *tert*-butyl alcohol to give the 2,2-disubstituted cyclohexanone **29** in 15% yield. For conversion of **29** into 17-benzylidene-*O*-methyl-*D*-homoestrone **32,** which has been transformed to estrone **3** by Johnson,[17] **29** was heated at 180 °C for 16 h in *o*-dichlorobenzene. However, the product was not the expected compound **32** but 5,6,6a,7,8,9,10,11,12,12a-decahydro-6a,10-methano-3-methoxy-10-methyl-6-phenylcycloocta[*a*]naphthalen-13-one (**31**) formed by an intramolecular cycloaddition of *o*-quinodimethane with the olefinic system in the α,β-unsaturated ketone in **30,** whose structure was determined by IR ($CHCl_3$), showing a saturated six-membered ketone at 1706 cm^{-1}, and NMR spectra (CCl_4), revealing a vinyl group at 4.60–5.23 ppm as a multiplet.

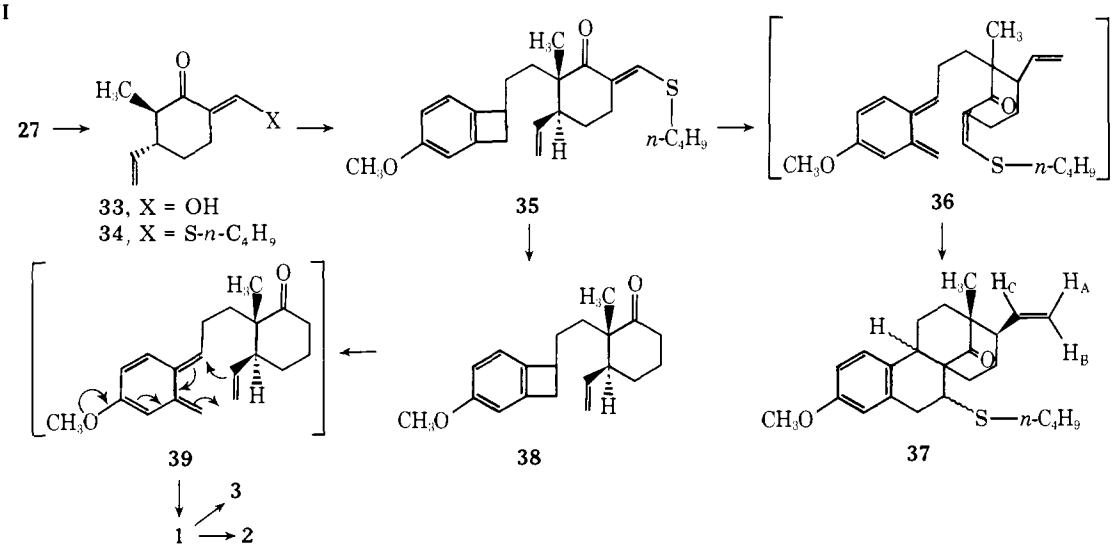
As numerous attempts to convert **29** into the *D*-homoestrone **32** under several conditions failed, our attention then turned to an intramolecular cycloaddition of 2-[2-(4-methoxybenzocyclobutenyl)ethyl]-2-methyl-3-vinylcyclohexanone (**38**).

Condensation of **27** with ethyl formate was achieved in the presence of sodium hydride in benzene and the resulting crude 6-hydroxymethylene derivative **33** was converted into 6-*n*-butylthiomethylenecyclohexanone **34** by treatment with *n*-butyl mercaptan and *p*-toluenesulfonic acid in benzene.[25] Alkylation of the cyclohexanone **34** with the iodide **17** in the presence of potassium *tert*-butoxide in *tert*-butyl alcohol, initially at 20 °C followed by heating to 80 °C, afforded stereoselectively the 2,2-disubstituted cyclohexanone derivative **35** in 16% yield by an attack of **17** on the less hindered side of **34**. Further experiments were performed in an effort to elevate the yield of **35** under several reaction conditions such as using other bases or at different reaction temperatures, but no in-

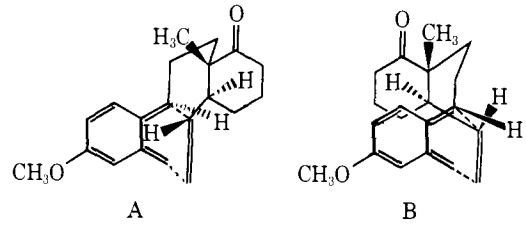
Scheme V



Scheme VI



Scheme VII



creasing of yield was observed. Heating **35** in *o*-dichlorobenzene at 180 °C overnight resulted in a cycloaddition between the *o*-quinodimethane part and the α,β-unsaturated carbonyl system in **36** to give the cyclized product **37,** which showed the saturated six-membered ketone at 1705 cm^{-1} in the IR spectrum ($CHCl_3$) and revealed olefinic protons at 4.90 (dd, J = 2 and 17 Hz, H_A), 4.95 (dd, J = 2 and 10 Hz, H_B) and 5.73 (dd, J = 10 and 17 Hz, H_C) in the NMR spectrum (CCl_4). The appearance of the vinyl protons of **37** at a higher field than those of **35** suggested that the relative configuration between the 2-methyl and the 3-vinyl groups would be cis because the vinyl group in **37** was located over the carbonyl group in the cis, but not in the trans, configuration, based on a consideration of Dreiding models. Removal of the protecting group in **35** was achieved successfully in 44.5% yield using potassium hydroxide[25] in ethylene glycol at 100 °C to afford the key intermediate **38** having a correct stereochemistry in a relative configuration between C_2 methyl and C_3 hydrogen on the cyclohexanone ring. Direct synthesis of **38** from **26** by a trapping method[22] was tried but resulted in failure.

Thermolysis of **38** proceeded smoothly in boiling *o*-dichlorobenzene for 4 h to afford *O*-methyl-*D*-homoestrone (**1**) in 95% yield in a regioselective and stereoselective manner. The IR ($CHCl_3$) and NMR (CCl_4) spectra of our product were superimposable upon those of an authentic sample prepared from natural estrone.[28] The stereocontrolled formation of **1** can be explained as follows. The four-membered ring in **38** opens to form preferentially the sterically favored *E*-oriented *o*-quinodimethane **39**,[14] whose synchronous cycloaddition reaction with vinyl group proceeds regiospecifically through the more stable exo transition state A rather than endo state B which has steric repulsion between the aromatic and the cyclohexanone ring.

Finally, demethylation[15] of **1** with pyridine hydrochloride as usual gave *D*-homoestrone (**2**). *O*-Methyl-*D*-homoestrone (**1**) has previously been correlated to estrone (**3**),[15-17] so this work constitutes a total synthesis of estrone.

Our synthetic method of *D*-homoestrone provides a new

approach based on an effective stereoselective cycloaddition reaction and has a high possibility as a general synthetic method for A-aromatized steroids.

Experimental Section

General. All melting points are uncorrected. Infrared spectra were recorded on a Hitachi EPI-3 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were measured on a JEOL-JNM-PMX-60 spectrometer. Chemical shifts are reported as δ values relative to internal tetramethylsilane (Me_4Si). Mass spectra were taken on a Hitachi RMU-7 spectrometer operating at an ionizing potential of 80 eV.

4-Methoxybenzocyclobutene-1-carboxylic Acid (8). To a solution of 4 g of KOH in 24 mL of EtOH was added 4.55 g of 1-cyano-4-methoxybenzocyclobutene (**7**). After the resulting solution was stirred for 13 h at room temperature and refluxed for 3 h, 200 mL of water was added and extracted with ether. The aqueous layer was acidified with 10% HCl and extracted with ether. The organic layer was washed with water and then with saturated NaCl solution and was dried over anhydrous sodium sulfate. Removal of the solvent afforded a colorless powder, which was recrystallized from hexane to give 4.1 g (80.4%) of carboxylic acid **8** as colorless needles: mp 85–86 °C; IR ν_{max} ($CHCl_3$) 1700 cm^{-1}; NMR (CCl_4) δ 3.38 (2 H, d, J = 4 Hz, $-CH_2CO_2H$), 3.75 (3 H, s, OCH_3), 4.18 (1 H, t, J = 4 Hz, C_1H), and 6.53–7.10 (3 H, m, ArH); m/e 178 (M^+). Anal. ($C_{10}H_{10}O_3$) C, H.

2-(4-Methoxybenzocyclobutenyl)methyl *p*-Toluenesulfonate (10). To a suspension of 4 g of lithium aluminum hydride in 80 mL of anhydrous THF was added a solution of 12.6 g of carboxylic acid (**8**) in 80 mL of anhydrous THF under stirring and then the solution was stirred for 6 h at room temperature. After addition of 10% NaOH aqueous solution, filtration of the inorganic compound, and evaporation of THF, the aqueous layer was extracted with ether. The ethereal layer was washed with water and saturated NaCl solution and then dried over anhydrous sodium sulfate. Evaporation of the solvent afforded 11.6 g of alcohol **9** as a yellow oil: NMR ($CDCl_3$) δ 2.29 (1 H, broad s, OH), 2.82 (1 H, dd, J = 1.5, 14 Hz, C_2 H), 3.27 (1 H, dd, J = 5, 14 Hz), 3.48–4.03 (1 H, m, C_2 H), 3.74 (3 H, s, OCH_3), and 6.63–7.20 (3 H, m, ArH); m/e 164 (M^+).

A solution of 11.6 g of alcohol **9** and 24.8 g of *p*-toluenesulfonyl chloride in 150 mL of pyridine was stirred for 13 h at room temperature. The reaction mixture was poured into 10% HCl solution under ice cooling and the crystals deposited were recrystallized from MeOH to give 16.9 g (76%) of tosylate **10** as colorless needles: mp 60–63 °C; NMR ($CDCl_3$) δ 2.45 (3 H, s, $-CH_3$), 2.75 (1 H, dd, J = 3, 13.5 Hz, C_2 H), 3.28 (1 H, dd, J = 5, 13.5 Hz, C_2 H), 3.75 (3 H, s, OCH_3), 4.10–4.43 (2 H, m, $-CH_2OTs$), 6.58–7.02 (3 H, m, ArH), 7.32 (2 H, d, J = 8 Hz, ArH), and 7.79 (2 H, d, J = 8 Hz, ArH); m/e 318 (M^+). Anal. ($C_{17}H_{18}O_4S$) C, H.

2-(4-Methoxybenzocyclobutenyl)methyl Cyanide (11). To a solution of 5.1 g of sodium cyanide in 52 mL of Me_2SO was added a solution of 16.9 g of tosylate **10** in 88 mL of Me_2SO and the solution was stirred for 13 h at room temperature. The resulting reaction mixture was poured into 1 L of water and extracted with ether. The ethereal layer was washed with water and saturated NaCl solution and dried over anhydrous sodium sulfate. Evaporation of the solvent afforded 9.9 g of cyanide **11** as a yellow oil: IR ν_{max} ($CHCl_3$) 2250 cm^{-1}; NMR ($CDCl_3$) δ 2.71 (2 H, d, J = 7 Hz, $-CH_2CN$), 2.80–3.73 (3 H, m, C_1 H, C_2 H_2), 3.75 (3 H, s, OCH_3), and 6.57–7.23 (3 H, m, ArH); m/e 173 (M^+).

2-(4-Methoxybenzocyclobutenyl)acetic Acid (12). A solution containing 8 g of crude nitrile **11** and 18.9 g of KOH in 65 mL of EtOH was stirred for 13 h and after addition of 21 mL of water, the resulting mixture was refluxed for 3 h. After evaporation of EtOH, 330 mL of water was added and extracted with ether. The aqueous phase was acidified with 10% HCl solution and extracted with ether. The ethereal extract was washed with water and saturated NaCl solution and then dried over anhydrous sodium sulfate. Evaporation of the solvent gave a white solid, which was recrystallized from benzene–hexane to give 7.4 g (83% from **10**) of carboxylic acid **12** as colorless needles: mp 62–65 °C; IR ν_{max} ($CHCl_3$) 1706 cm^{-1}; NMR (CCl_4) δ 2.71 (2 H, d, J = 7 Hz, $-CH_2CO_2H$), 2.78–3.68 (3 H, m, C_1 H, C_2 H_2), 3.70 (3 H, s, OCH_3), and 6.33–7.10 (3 H, m, ArH); m/e 192 (M^+). Anal. ($C_{11}H_{12}O_3$) C, H.

2-(4-Methoxybenzocyclobutenyl)ethanol (13). To a slurry of 4.2 g of lithium aluminum hydride in 110 mL of anhydrous THF was added 14.8 g of carboxylic acid **12** in 100 mL of anhydrous THF and the solution was stirred for 13 h at room temperature. After quenching with 30% aqueous NaOH solution, filtration of inorganic compound, and evaporation of THF, the residue was extracted with ether. The ethereal extract was washed with water and saturated NaCl solution and dried over anhydrous sodium sulfate. Evaporation of ether afforded 13.5 g of alcohol **13** as a yellow oil: NMR (CCl_4) δ 1.56–2.06 (2, H, m, $-CH_2CH_2OH$), 2.43–3.53 (3 H, m, C_1 H, C_2 H_2), 3.53–3.80 (2 H, m, $-CH_2CH_2OH$), 3.63 (3 H, s, OCH_3), and 6.46–6.88 (3 H, m, ArH); m/e 178 (M^+).

2-(4-Methoxybenzocyclobutenyl)ethyl *p*-Toluenesulfonate (16). To a solution of 13.5 g of alcohol **13** in 60 mL of pyridine was added 27.3 g of *p*-toluenesulfonyl chloride and the solution was stirred for 13 h at room temperature. The resulting reaction mixture was poured into 10% HCl solution and extracted with ether. The ethereal phase was washed with water and saturated NaCl solution. After drying over anhydrous sodium sulfate, the organic layer was removed and the residue was chromatographed on 300 g of silica gel (using benzene for elution) to afford colorless crystals, which were recrystallized from MeOH to give 11.3 g (44% from **12**) of tosylate **16**, mp 61–64 °C, as colorless needles: NMR (CCl_4) δ 1.70–2.14 (2 H, m, $-CH_2CH_2OTs$), 2.39 (3 H, s, $-CH_3$), 2.60–3.50 (3 H, m, C_1 H, C_2 H_2), 3.63 (3 H, s, OCH_3), 4.07 (2 H, t, J = 7 Hz, $-CH_2CH_2OTs$), 6.44–6.87 (3 H, m, ArH), and 7.14–7.84 (4 H, m, ArH); m/e 332 (M^+). Anal. ($C_{18}H_{20}O_4S$) C, H.

2-(1-Cyano-4-methoxybenzocyclobutenyl)ethyl Pyranyl Ether (14). To a stirred solution of 1.59 g of benzocyclobutene (**7**) and sodium amide (prepared from 260 mg of sodium) in liquid ammonia was added 2.09 g of 2-(2-bromoethoxy)tetrahydropyran in 5 mL of anhydrous THF dropwise at −70 °C. After stirring was continued for 30 min at the same temperature, the reaction mixture was treated with an excess of crystalline ammonium chloride and the solvent was removed to give a reddish residue, which was diluted with 20 mL of saturated aqueous ammonium chloride solution. The resulting mixture was extracted with ether, and the ethereal extract was washed with saturated NaCl solution and dried over anhydrous sodium sulfate. Removal of the solvent afforded a reddish gum, which was chromatographed on 25 g of silica gel (using hexane–benzene for elution) to give 2.47 g (86%) of pyranyl ether **14** as a colorless oil: IR ν_{max} ($CHCl_3$) 2225 cm^{-1}; NMR ($CDCl_3$) δ 1.3–1.9 (6 H, m), 2.15 (2 H, t, J = 6 Hz, $-CH_2CH_2O-$), 3.15–4.2 (6 H, m), 3.73 (3 H, s, OCH_3), 4.57 (1 H, broad s, $-OCHO$), and 6.5–7.15 (3 H, m, ArH); m/e 287 (M^+).

2-(4-Methoxybenzocyclobutenyl)ethyl Pyranyl Ether (15). To a stirred solution containing 600 mg of benzocyclobutene (**14**), 1 mL of anhydrous 2-propanol, 10 mL of anhydrous THF, and 50 mL of liquid ammonia was added 50 mg of sodium at −70 °C and the solution was stirred for 30 min at the same temperature. After addition of an excess of crystalline ammonium chloride and evaporation of the solvent, the residue was diluted with saturated aqueous ammonium chloride solution and extracted with ether. The ethereal layer was washed with saturated NaCl solution and dried over anhydrous sodium sulfate. Removal of the solvent afforded a reddish gum, which was chromatographed on 10 g of silica gel (using hexane–benzene for elution) to give 460 mg (84%) of **15** as a colorless syrup: NMR ($CDCl_3$) δ 1.4–2.3 (8 H, m), 2.5–4.1 (6 H, m), 3.7 (3 H, s, OCH_3), 4.6 (1 H, broad s, $-OCHO$), and 6.5–7.05 (3 H, m, ArH); m/e 262 (M^+).

2-(4-Methoxybenzocyclobutenyl)ethanol (13) from 15. The mixture of 150 mg of pyranyl ether **15**, 10 mL of MeOH, and 0.5 mL of 10% HCl solution was stirred for 3 h. After evaporation of the solvent, 10 mL of water was added and the solution was extracted with ether. The ethereal extract was washed with saturated NaCl solution and dried over anhydrous sodium sulfate. Removal of the solvent gave a yellow oil, which was chromatographed on 10 g of silica gel (using hexane–benzene for elution) to afford 80 mg (93.9%) of **13** as a colorless oil. This was shown to be identical with the compound **13** obtained from the reduction of carboxylic acid **12** as described previously in its IR ($CHCl_3$) and NMR (CCl_4) spectra.

2-(4-Methoxybenzocyclobutenyl)ethyl Iodide (17). A solution containing 11.3 g of tosylate **16**, 15.6 g of sodium iodide, and 173 mL of acetone was refluxed for 3.5 h. After evaporation of the solvent, 100 mL of water was added and the solution was extracted with ether. The ethereal extract was washed with 5% $Na_2S_2O_3$ aqueous solution and saturated NaCl solution and dried over anhydrous sodium sulfate. After removal of the solvent, the residue was chromatographed on 100

g of silica gel (using hexane–benzene for elution) to give 9.0 g (92%) of iodide **17** as a colorless oil: NMR (CCl_4) δ 1.97–2.41 (2 H, m, $-CH_2CH_2I$), 2.44–3.64 (5 H, m, $-CH_2CH_2I$, C$_1$ H, C$_2$ H$_2$), 3.61 (3 H, s, OCH_3), and 6.50–7.04 (3 H, m, ArH); *m/e* 288 (M^+).

3-Ethenyl-2-methylcyclopentan-1-one (19). To a slurry containing 16 g of cuprous iodide, vinylmagnesium bromide (prepared from 6 g of magnesium and 26 g of vinyl bromide), and 300 mL of anhydrous THF under an atmosphere of nitrogen was added dropwise 4 g of 2-methylcyclopent-2-en-1-one in 50 mL of anhydrous THF at −70 °C and the solution was stirred for 30 min at the same temperature. After addition of saturated aqueous ammonium chloride, the inorganic compound was filtered off and the filtrate was extracted with ether. The ethereal extract was washed with saturated aqueous ammonium chloride and dried over anhydrous sodium sulfate. Removal of the solvent afforded a yellow oil, which was distilled in vacuo to give 2.1 g (41%) of **19** as a colorless oil: bp 74–76 °C (30 mm); IR ν_{max} ($CHCl_3$) 1730 cm^{-1}; NMR (CCl_4) δ 0.9 (3 H, d, J = 8 Hz, $-CH_3$), 1.5–3.3 (6 H, m), and 4.7–6.0 (3 H, m, $HC{=}CH_2$); *m/e* 124 (M^+).

5-*n*-Butylthiomethylene-3-ethenyl-2-methylcyclopentanone (21). To a solution of 5 g of pentanone **19** add 13.32 g of ethyl formate in 100 mL of anhydrous benzene was added in small portions 8.64 g of sodium hydride (50% in oil) and the solution was stirred for 30 min at room temperature. After addition of 100 mL of water, the organic layer was washed with 50 mL of 10% aqueous NaOH solution. The combined aqueous solution was acidified with 10% aqueous H_2SO_4 solution and extracted with ether. The ethereal extract was washed with saturated NaCl solution and dried over anhydrous sodium sulfate. Evaporation of the solvent afforded 3.81 g of the crude hydroxymethylene compound (**20**), which was used for the next reaction without purification. Thus a solution containing 3.81 g of crude compound **20**, 6 g of *n*-butyl mercaptan, a catalytic amount of *p*-toluenesulfonic acid, and 100 mL of anhydrous benzene was refluxed under an atmosphere of nitrogen for 1 h. After cooling to room temperature, 100 mL of saturated aqueous $NaHCO_3$ solution was added. The resulting mixture was extracted with ether and the organic extract was washed with water and saturated NaCl solution and finally dried over anhydrous sodium sulfate. After removal of the solvent, the residue was distilled in vacuo to give 1.3 g (13.44%) of **21** as a colorless oil: bp 68–70 °C (5 mmHg); IR ν_{max} ($CHCl_3$) 1690 cm^{-1}; NMR (CCl_4) δ 0.9–3.1 (16 H, m), 4.9–6.1 (3 H, m, $HC{=}CH_2$), and 7.18 (1 H, broad s, $=CHS$-*n*-Bu); *m/e* 224 (M^+).

5-*n*-Butylthiomethylene-3-ethenyl-2-[2-(4-methoxybenzocyclobutenyl)ethyl]-2-methylcyclopentanone (22). To a solution containing 169 mg of potassium *tert*-butoxide, 4 mL of hexamethylphosphoryl triamide, and 10 mL of anhydrous THF under an atmosphere of nitrogen was added dropwise 470 mg of **21** at −20 °C. After stirring was continued for 20 min at the same temperature, 1.22 g of iodide **17** in 3 mL of anhydrous THF was added and the solution was stirred for 5 h at the same temperature. To this reaction mixture 20 mL of water was added and the resulting mixture was extracted with ether. The ethereal extract was washed with saturated NaCl solution and dried over anhydrous sodium sulfate. Evaporation of the solvent left a brown oil, which was chromatographed on 20 g of silica gel (using hexane–benzene for elution) to give 205 mg of **22** as a colorless oil: IR ν_{max} ($CHCl_3$) 1690 cm^{-1}; NMR (CCl_4) δ 0.85 (3 H, s, $-CH_3$), 3.70 (3 H, s, OCH_3), 4.85–5.9 (3 H, m, $HC{=}CH_2$) 6.45–7.0 (3 H, m, ArH), and 7.3 (1 H, broad s, $=CHS$-*n*-Bu); *m/e* 384 (M^+).

3-Ethenyl-2-methylcyclohexanone (27). To a slurry of 60 g of cuprous iodide and vinylmagnesium bromide (prepared from 7.3 g of magnesium and 33 g of vinyl bromide) under an atmosphere of nitrogen was added 15 g of 2-methylcyclohex-2-en-1-one (**26**) in anhydrous THF at −70 °C, and the solution was worked up as in **19** to give 12.4 g (66%) of **27** as a colorless oil: bp 63–65 °C (4 mm); IR ν_{max} ($CHCl_3$) 1700 cm^{-1}; (CCl_4) δ 0.91 (3 H, d, J = 4.6 Hz, $-CH_3$) and 4.77–5.93 (3 H, m, $HC{=}CH_2$); *m/e* 138 (M^+).

6-Benzal-3-ethenyl-2-methylcyclohexanone (28). A solution containing 3.2 g of **27**, 3.5 mL of benzaldehyde, 14 mL of water, 1.5 g of NaOH, and 9 mL of EtOH was refluxed for 4.5 h. After cooling to room temperature, the reaction mixture was extracted with ether, and the ethereal layer was washed with saturated NaCl solution and dried over anhydrous sodium sulfate. Evaporation of the solvent afforded yellow crystals,which were recrystallized from MeOH to give 1.9 g (36.5%) of **28**, mp 70–72 °C, as colorless prisms: IR ν_{max} ($CHCl_3$) 1674 cm^{-1}; NMR (CCl_4) δ 1.15 (3 H, d, J = 6 Hz, $-CH_3$), 4.8–6.0 (3 H, m, $HC{=}CH_2$), and 7.25 (6 H, broad s, $=CHC_6H_5$); *m/e* 226 (M^+). Anal. ($C_{16}H_{18}O$) C, H.

6-Benzal-3-ethenyl-2-[2-(4-methoxybenzocyclobutenyl)-ethyl]-2-methylcyclohexanone (29). To a solution of potassium *tert*-butoxide (prepared from 275 mg of potassium) in 20 mL of *tert*-butyl alcohol under an atmosphere of nitrogen was added 1.3 g of **28** in 10 mL of *tert*-butyl alcohol. After stirring was continued for 1 h at room temperature, 1.7 g of iodide **17** in 10 mL of *tert*-butyl alcohol was added and the resulting mixture was stirred for 13 h at the same temperature. The solvent was distilled off under reduced pressure, and the residue was diluted with 20 mL of water and acidified with 10% HCl solution. The resulting mixture was extracted with ether and the ethereal extract was washed with saturated NaCl solution and dried over anhydrous sodium sulfate. Evaporation of the solvent left a yellow oil, which was chromatographed on 30 g of silica gel (using hexane–benzene for elution) to afford 346 mg (15%) of **29** as a colorless oil: IR ν_{max} ($CHCl_3$) 1670 cm^{-1}; NMR (CCl_4) δ 0.99 (3 H, s, $-CH_3$), 3.65 (3 H, s, OCH_3), 4.77–6.00 (3 H, m, $HC{=}CH_2$), and 6.47–7.74 (9 H, m, $=CHC_6H_5$, C$_3$ H, C$_5$ H, C$_6$ H); *m/e* 386 (M^+).

Thermolysis of Compound 29. A solution of 53 mg of **29** in 5 mL of *o*-dichlorobenzene was stirred for 13 h at 180 °C under an atmosphere of nitrogen. After evaporation of the solvent, the residue was recrystallized from EtOH to give 8.8 mg (16.6%) of 9-ethenyl-5,6,6a,7,8,9,10,11,12,12a-decahydro-6a,10-methano-3-methoxy-10-methyl-6-phenylcycloocta[*a*]naphthalen-13-one (**31**), mp 142–144 °C, as colorless plates: IR ν_{max} ($CHCl_3$) 1706 cm^{-1}; NMR (CCl_4) δ 0.86 (3 H, s, $-CH_3$), 3.78 (3 H, s, OCH_3), 4.60–5.23 (3 H, m, $HC{=}CH_2$), and 6.48–7.70 (8 H, m, ArH); *m/e* 386 (M^+). Anal. ($C_{27}H_{30}O_2$) C, H.

6-*n*-Butylthiomethylene-3-ethenyl-2-methylcyclohexanone (34). To a solution of 6.7 g of **27** and 4.3 g of ethyl formate in 50 mL of anhydrous benzene was added in small portions 7.0 g of sodium hydride (50% in oil) and the mixture was stirred for 30 min at room temperature. Workup of this mixture afforded 6.3 g of crude **33** as a yellow oil, which was used for the next reaction without further purification. A solution of 2 g of crude **33**, 1.1 g of *n*-butyl mercaptan, and a catalytic amount of *p*-toluenesulfonic acid in 50 mL of anhydrous benzene was refluxed under an atmosphere of nitrogen for 1 h. The same treatment as for **21** gave 1.8 g (62%) of **34** as a colorless oil: bp 155–160 °C (5 mm); IR ν_{max} ($CHCl_3$) 1645 cm^{-1}; NMR (CCl_4) δ 0.97 (3 H, d, J = 6 Hz, $-CH_3$), 4.77–6.00 (3 H, m, $HC{=}CH_2$), and 7.30 (1 H, broad s, $=CHS$-*n*-Bu); *m/e* 238 (M^+). Anal. ($C_{14}H_{22}OS$) C, H.

6-*n*-Butylthiomethylene-3-ethenyl-2-[2-(4-methoxybenzocyclobutenyl)ethyl]-2-methylcyclohexanone (35). To a solution of potassium *tert*-butoxide (prepared from 50 mg of potassium) in 30 mL of *tert*-butyl alcohol under an atmosphere of nitrogen was added 2 g of **34** in 10 mL of *tert*-butyl alcohol at room temperature. After stirring was continued for 1 h at the same temperature, 859 mg of the iodide **17** was added and then the solution was stirred for 13 h at the same temperature. Same workup of this mixture as for **22** left the residue, which was chromatographed on 50 g of silica gel (using hexane–benzene for elution) to give 190 mg (16%) of **35** as a colorless oil: IR ν_{max} ($CHCl_3$) 1645 cm^{-1}; NMR (CCl_4) δ 0.95 (3 H, s, $-CH_3$), 3.68 (3 H, s, OCH_3), 5.00 (1 H, dd, J = 2, 17 Hz, $HHC{=}CH$), 5.03 (1 H, dd, J = 2, 8.5 Hz, $HHC{=}CH$), 5.75 (1 H, distorted dd, J = 8.5, 17 Hz, $HC{=}CH_2$), 6.48–6.73 (2 H, m, C$_3$ H, C$_5$ H), 6.88 (1 H, d, J = 9 Hz, C$_6$ H), and 7.35 (1 H, broad s, $=CHS$-*n*-Bu); *m/e* 398 (M^+). Anal. ($C_{25}H_{34}O_2S{\cdot}0.5H_2O$) C, H.

Thermolysis of Compound 35. A solution of 80 mg of **35** in 10 mL of *o*-dichlorobenzene was stirred under an atmosphere of nitrogen for 13 h at 180 °C. After evaporation of the solvent, the residue was chromatographed on 2 g of silica gel (using hexane–benzene for elution) to give 30.3 mg (38%) of 6-*n*-butylthio-9-ethenyl-5,6,6a,7,8,9,10,11,12,12a-decahydro-6a,10-methano-3-methoxy-10-methylcycloocta[*a*]naphthalen-13-one (**37**) as a colorless oil: IR ν_{max} ($CHCl_3$) 1705 cm^{-1}; NMR (CCl_4) δ 0.92 (3 H, s, $-CH_3$), 3.68 (3 H, s, OCH_3), 4.90 (1 H, dd, J = 2, 17 Hz, $HH_tC{=}CH$), 4.95 (1 H, dd, J = 2, 10 Hz, $H_cHC{=}CH$), 5.73 (1 H, m, J = 10, 17 Hz, $HC{=}CH_2$), 6.43–6.65 (2 H, m, C$_2$ H, C$_4$ H), 6.93 (1 H, d, J = 8 Hz, C$_1$ H); *m/e* 398 (M^+). Anal. ($C_{25}H_{34}O_2S{\cdot}\frac{5}{6}H_2O$) C, H.

3-Ethenyl-2-[2-(4-methoxybenzocyclobutenyl)ethyl]-2-methylcyclohexanone (38). A solution of 90 mg of **35** and 1 mL of 25% aqueous KOH solution in 1.5 mL of diethylene glycol was stirred under an atmosphere of nitrogen for 13 h at 100 °C. The cooled reaction mixture was extracted with benzene, and the benzene extract was washed with water and dried over anhydrous sodium sulfate. After

removal of the solvent, the residue was chromatographed on 1 g of silica gel (using hexane–benzene for elution) to afford 30 mg (44.5%) of **38** as a colorless oil: IR ν_{max} ($CHCl_3$) 1690 cm^{-1}; NMR (CCl_4) δ 0.96 (3 H, s, $-CH_3$), 3.70 (3 H, s, OCH_3) 4.78–5.92 (3 H, m, $HC{=}CH_2$) 6.43–6.67 (2 H, m, C_3 H, C_5 H), and 6.83 (1 H, d, J = 8 Hz, C_6 H); *m/e* 298 (M^+). Anal. ($C_{20}H_{26}O_2 \cdot 0.5H_2O$) C, H.

Thermolysis of Compound 38. A solution of 30 mg of **38** in 4 mL of *o*-dichlorobenzene was stirred under an atmosphere of nitrogen for 4 h at 180 °C. After evaporation of the solvent, the residue was recrystallized from ethyl acetate to give 28.6 mg (95.3%) of *D*-homoestrone methyl ether (**1**); mp 160–162 °C (lit.[15,16] mp 155–157 °C, 158–160 °C, 162–163 °C), as colorless prisms: IR ν_{max} ($CHCl_3$) 1693 cm^{-1}; NMR (CCl_4) δ 1.08 (3 H, s, $-CH_3$), 3.69 (3 H, s, OCH_3), 6.39–6.72 (2 H, m, C_2H), 7.08 (1 H, d, J = 9 Hz, C_1 H); *m/e* 298 (M^+). Anal. ($C_{20}H_{26}O_2 \cdot \frac{1}{6}H_2O$) C, H. This was shown to be identical with the authentic sample in its IR ($CHCl_3$) and NMR (CCl_4) spectra.

***D*-Homoestrone (2).** A mixture of 10 mg of **1** and 500 mg of freshly prepared dry pyridine hydrochloride was heated at 200 °C for 40 min under an atmosphere of nitrogen. After cooling to room temperature, 2 mL of 5% HCl solution was added. The aqueous solution was extracted with ether and the ethereal extract was washed with water, saturated $NaHCO_3$ solution, and saturated NaCl solution, and finally dried over anhydrous sodium sulfate. After evaporation of the solvent, the residue was recrystallized from hexane–ether to give 7.6 mg (80%) of **2** as colorless plates, mp 218–222 °C (lit.[6] 220–223 °C).

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Molecular Orbital Studies of Enzyme Activity. 4. Hydrolysis of Peptides by Carboxypeptidase A

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Abstract: An approximate molecular orbital method is used to study the catalytic activity of carboxypeptidase A. A proton donor is positioned near the nitrogen atom of the scissile bond of a model substrate. Nucleophilic attack on the carbonyl carbon leads to hydrolysis of the peptide. An electrophile positioned proximate to the carbonyl oxygen is seen to greatly facilitate this hydrolysis. The electrophile first polarizes the carbonyl bond of the substrate, making the carbon more susceptible to nucleophilic attack. The tetrahedral adduct formed as a result of this attack is stabilized by the electrophile in several ways. Bonding between the electrophile and the carbonyl oxygen reduces the negative charge on the oxygen. The electrophile also acts to strengthen the bonding between the central carbon and its four substituents in the adduct. The metal electrophiles which model Zn^{2+} and its ligands are more effective at catalyzing the hydrolysis than are various hydrogen-bonding species. The effects of strain on the peptide bond as a result of binding to the enzyme are examined for each of the electrophiles. Finally, a comparison is made between several proposed modes of nucleophilic attack.

Carboxypeptidase A (CPA) catalyzes the hydrolysis of carboxy-terminal peptide bonds. CPA is a metalloenzyme in which the presence of Zn^{2+} is a cofactor for enzyme activity.[1] Peptidase activity has been observed when Zn^{2+} is replaced by Co^{2+}, Ni^{2+}, Mn^{2+}, and Fe^{2+}.[1-3] Substitution by Hg^{2+}, Cd^{2+}, and Pb^{2+} results in loss of peptidase activity, although these heavy metal derivatives retain esterase activity.[3,4] In Zn-CPA, the Zn^{2+} is coordinated to His-69, His-196, Glu-72, and one water molecule in a distorted tetrahedral configuration about the zinc.[5] On binding of glycyl-L-tyrosine[5] the zinc-coordinated water molecule is displaced by the carbonyl oxygen of the scissile peptide bond. The C-terminal carboxylate group