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Chromogenic Reactions of Steroids with Strong Acids. XI.[1] Mechanism of Chromogenic Reaction of Testosterone with Sulfuric Acid

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Mechanism was investigated for the chromogenic reaction of testosterone (I) with sulfuric acid. Dication (II in Chart 1) produced at the primary stage of the reaction was transformed into the chromophoric χ-484 (XIIa in Chart 4) *via* its conjugate base (VIII in Chart 4) by acid-base catalyzed isomerization. The conjugate base of the χ-484 (XIIb), 17,17-dimethyl-18-norandrosta-4,6,8(14)-trien-3-one (XIb), was isolated in 40% yield in the reaction of the epoxide (V in Chart 2) with sulfuric acid. Sulfuric acid solution of the trienone (XIa, b) showed a maximum absorption at 484 nm (ε=42000). The pK_a value of XIIa was also determined.

Keywords——testosterone; sulfuric acid; color reaction; dication; hydroxyalkatrienyl cation; pK_a of carbocation

The chromogenic reactions of steroids with strong acids have long been used for quantitative analyses and structure elucidations. Although the reactions of steroidal 4-en-3-ones such as testosterone (I) and corticoids with strong acids have fairly been investigated,[3] the mechanism still remains obscure. In our preceding paper,[1] the behavior of I and its related compounds in concentrated sulfuric acid was reported and the initial process was accounted for the chromogenic reaction of I(Chart 1). In this report, the formation mechanism and the chemical structure of the chromophoric species showing the maximum absorption at 484 nm are discussed, which is one of the two main chromophoric species produced in the reaction of I with sulfuric acid.

Results and Discussion

In the reactions of I with strong acids, two main chromophoric species, χ-484 and χ-600-(designation for the species indicating a maximum absorption at 484 and 600 nm, respectively) were produced. Since the dication (II, abbreviated as χ-300) was previously reported to be produced from I by protonation, dehydration, angular methyl migration, and oxidation at the early period in the chromogenic reaction and since it was assumed to be the essential intermediate to the chromophoric χ-484,[1] conversion of II into the χ-484 was the first objective of this study.

Dissolution of I into 97.2% sulfuric acid gave the χ-300(II) which was stable for a long time at room temperature as reported.[1] When the solution was diluted by pouring it into water to adjust the final concentration of sulfuric acid to 63.0%, the chromophoric χ-484 was produced as shown in Fig. 1. On the contrary, no visible absorption appeared when the solution was diluted in the reverse way. Although the final acid concentrations became

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[1] Part X: T. Miura, H. Takagi, K. Harita, and M. Kimura, *Chem. Pharm. Bull.* (Tokyo), **27**, 460 (1979).

[2] Location: *Nishi-6-chome, Kita-12-jo, Kita-ku, Sapporo, 060, Japan.*

[3] *a*) M. Kimura and K. Harita, *Chem. Pharm. Bull.* (Tokyo), **21**, 1205 (1973); *b*) *Idem, ibid.*, **21**, 1235 (1973); *c*) *Idem, ibid.*, **21**, 1261 (1973); *d*) H.A. Jones, *J. Chem. Soc.* (*B*), **1971**, 99; *e*) W. Sadée, S. Riegelman, and L.F. Johnson, *Steroids*, **17**, 595 (1971); *f*) A.R. Butler, *J. Chem. Soc., Perkin II*, **1976**, 959; *g*) A.R. Butler and H.A. Jones, *ibid.*, **1976**, 963; *h*) G. Tóth, A. Szabo, D. Müller, and G. Snatzke, *Collect. Czech. Chem. Commun.*, **43**, 165 (1978).

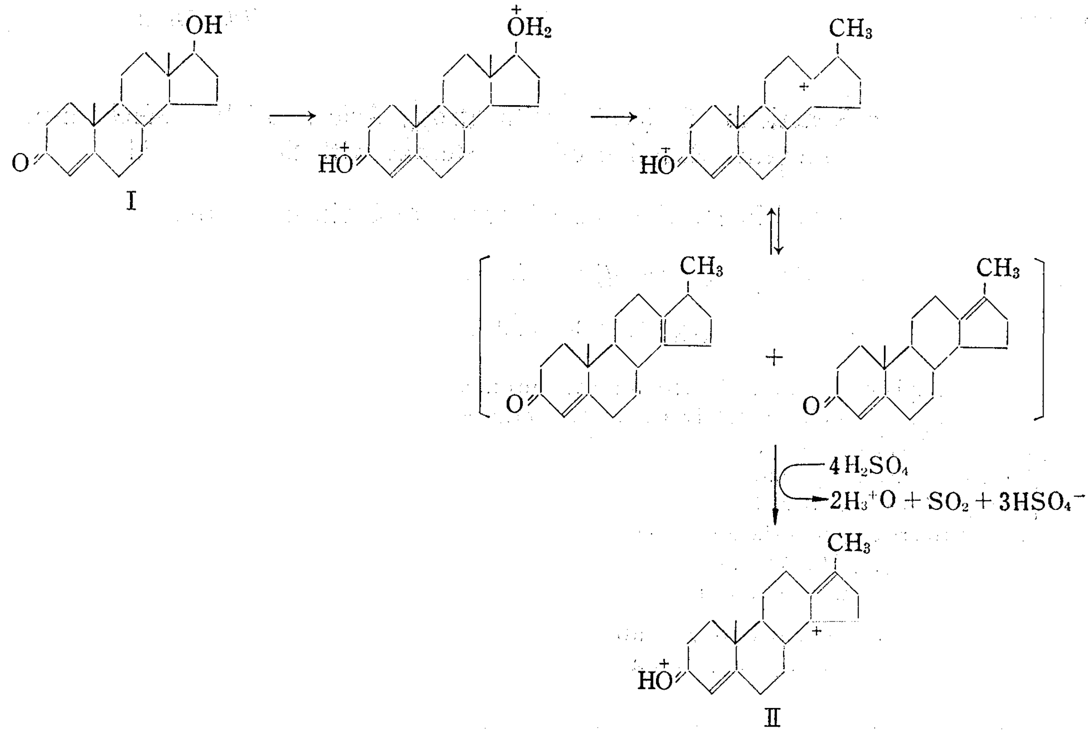


Chart 1. Primary Process of Chromogenic Reaction of Testosterone (I) with Sulfuric Acid

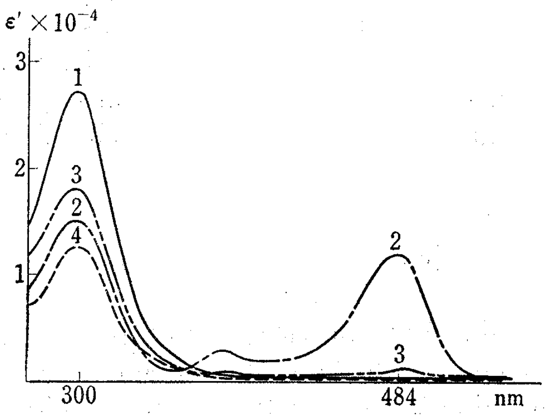


Fig. 1. Conversion of χ-300 (II) to Chromophoric χ-484 (XIIa)

1: 97.2% H_2SO_4 solution of I(28 μg/ml).
2: solution of 1 (3 ml) was poured into H_2O(3 ml).[a]
3: H_2O(3 ml) was poured into solution of 1 (3 ml).[a]
4: 63.0% H_2SO_4 solution of I(28 μg/ml).
a) Final concentration of H_2SO_4 of solutions of 2 and 3 is about 63%.

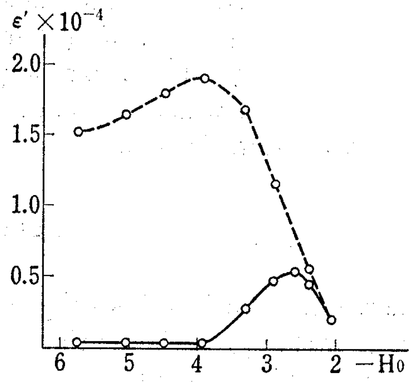


Fig. 2. Effect of Acid Strength on Formation of Chromophoric χ-484

Epoxide (40 μg) was dissolved into H_2SO_4 (5 ml) and absorbance at 484 nm was measured after standing for 10 min at room temperature.
——: III+IV, ------: V.

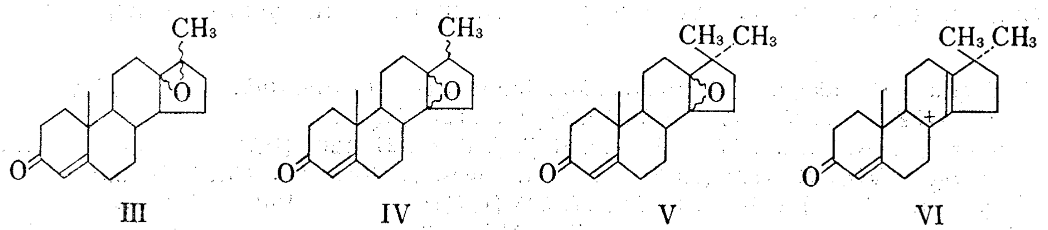
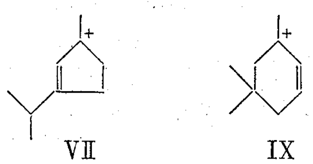


Chart 2

identical in these diluted solutions, II in the former was initially brought into contact with sulfuric acid of the concentration below 63.0%, contrary to that far more concentrated in the latter solution. It seemed, therefore, that the stability of the cation χ-300(II) depends on the acid strength of the medium and its subsequent reaction forming the chromophoric χ-484 occurs with decrease in the acid strength of the medium. In order to examine the effect of acid strength on the conversion of II to the χ-484, each of the epoxides (III+IV and V) which are the precursors(1) of II and its related cation (VI) respectively, was dissolved into aqueous sulfuric acids of various concentrations and the absorption intensity at 484 nm of the colored solution was measured after standing for 10 minutes at room temperature. The results obtained are shown in Fig. 2 where the Hammett's acidity function (H_0) is used as an index of the acid strength of medium. As demonstrated in Fig. 2, 1) the χ-300(II) prepared from the epoxide (a 1:1 mixture of III and IV) is stable in the aqueous sulfuric acid where $-H_0$ value is above 4,(4) 2) II is less stable and transformed into the χ-484 gradually when $-H_0$ value is approximately 3,(4) 3) the cation (VI) probably produced from the corresponding epoxide (V) was, on the other hand, labile and transformed into the χ-484 in sulfuric acid where $-H_0$ value is even above 5.(4) The stability of alkenyl cation is frequently expressed in the diene-alkenyl cation equilibrium constant; pK_a is based on H_0 and the following equation:(5)

$$H_0 = \mathrm{p}K_a + \log([C_B]/[C_{BH^+}])$$

The pK_a values of several alkenyl cations were measured and discussed in terms of the effect of structural changes on the stabilities of the cations.(5) In relation to the stability of II, 1-isopropyl-3-methylcyclopentenyl cation (VII) is of interest in view of its structural similarity to the alkenyl cation moiety of II. The pK_a value of VII was reported to be -2.6(5) and was in good agreement with the observed H_0 value, approximately -3, of the sulfuric acid where II was unstable and converted into the χ-484. This fact reveals that the χ-484 was formed from II *via* its conjugate base (VIII). Therefore, when the concentration of sulfuric acid in the solution of II was reduced to 63.0% ($H_0=-4.83$) by pouring it into water, a part of II was brought into contact with the acid of H_0 value above -3 and transformed into the χ-484. Through the reverse way of dilution, however, the concentration of the acid was held above 63.0% and II remained intact. On the other hand, the cation (VI) derived from the epoxide (V) is assumed to be less stable than II, since its pK_a value was inferred below -7 by referrence to that of -7.3 for 1,5,5-trimethylcyclohexenyl cation (IX).(5) Therefore, even at H_0 value below -5, VI was labile and converted into the χ-484 *via* its conjugate base (X).



VII IX

p$K_a=-2.60$ p$K_a=-7.30$

Chart 3

In order to elucidate the chemical structure of χ-484, the products were examined in the reaction of the epoxide (V) with sulfuric acid. When V was dissolved into 62.0% sulfuric acid and allowed to stand at room temperature for 5 minutes, 17,17-dimethyl-18-norandrosta-4,6,8(14)-trien-3-one (XIb) was obtained in 40% yield, which was confirmed by comparison of its spectral data with those of an authentic sample.(3e) Sadee *et al.* obtained the trienone (XIb) in the reaction of 17β-hydroxy-17α-methylandrosta-4,6-dien-3-one with 62% sulfuric acid and Toth *et al.* obtained the trienone (XIa) in the reaction of androst-4-ene-3,17-dione with concentrated sulfuric acid.(3h) After I was dissolved into 97.2% sulfuric acid and allowed to stand at room temperature for 15 minutes, the solution was poured into water and extracted with isooctane. The organic layer showed a characteristic absorption of XIa as shown in Fig. 3. From the maximum absorption intensity, 22% of I was assumed to be converted

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4) *cf.* H_0 values corresponding to the concentration (%) of H_2SO_4: $H_0=-5.02$ (64.5%); -4.09 (56.9); -3.33 (49.4); -2.90 (45.5).

5) N.C. Deno, J. Bollinger, K. Hafer, J.D. Hodge, and J.J. Houser, *J. Am. Chem. Soc.*, **85**, 2998 (1963).

into XIa under the condition. Dissolution of XIa into 65% sulfuric acid immediately showed a maximum absorption at 484 nm ($\varepsilon'=42000$) as shown in Fig. 4 and as has been observed by Sadee *et al.*[3e] Moreover, the maximum absorption was shifted from 348 nm ($\varepsilon'=29000$) to 475 nm ($\varepsilon'=48000$) on the addition of one drop of 70% perchloric acid to the dichloromethane solution of XIa. When the colored solution was shaken with water to remove the acid, the color of the organic layer faded and the characteristic absorption of XIa at 348 nm appeared again as shown in Fig. 4. This reversible change between XIa and the chromophoric χ-484 indicates that the χ-484 is the hydroxyalkatrienyl cation (XIIa) which was formed by the acid-catalized isomerization of II as shown in Chart 4.

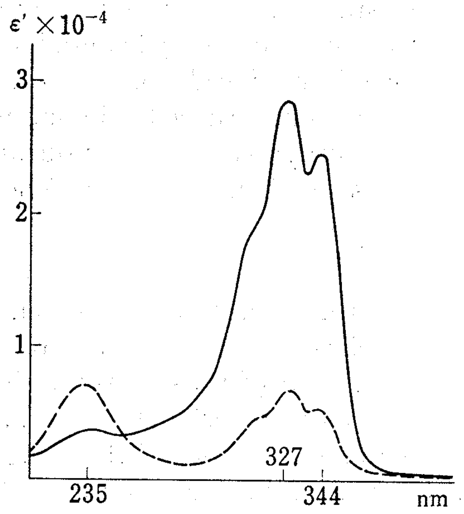
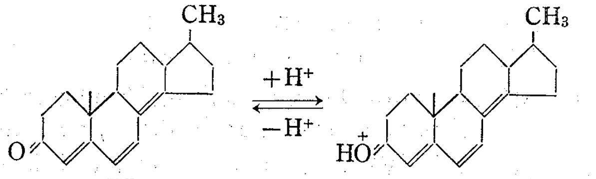


Fig. 3. 17ξ-Methyl-18-norandrosta-4,6,8(14)-trien-3-one (XIa) from Reaction Mixture of Testosterone (I) with Sulfuric Acid

210 μg of I was dissolved into 1.5 ml of 97.2% H_2SO_4, allowed to stand at room temperature for 15 min, poured into 2 ml of H_2O, and finally extracted with 4 ml of isooctane.
——: XIa, ------: isooctane extract.



XIa
348 nm (CH_2Cl_2)

XIIa
475 nm (70% $HClO_4$-CH_2Cl_2)
484 nm (65% H_2SO_4)

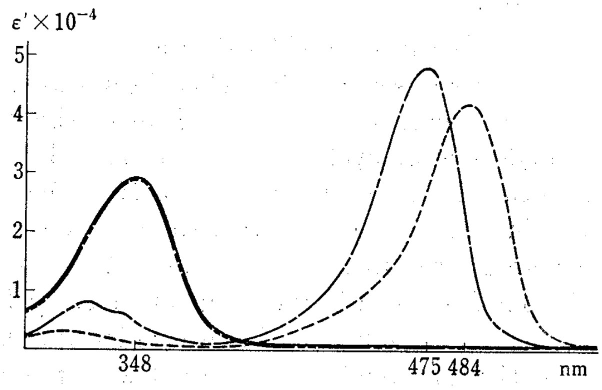
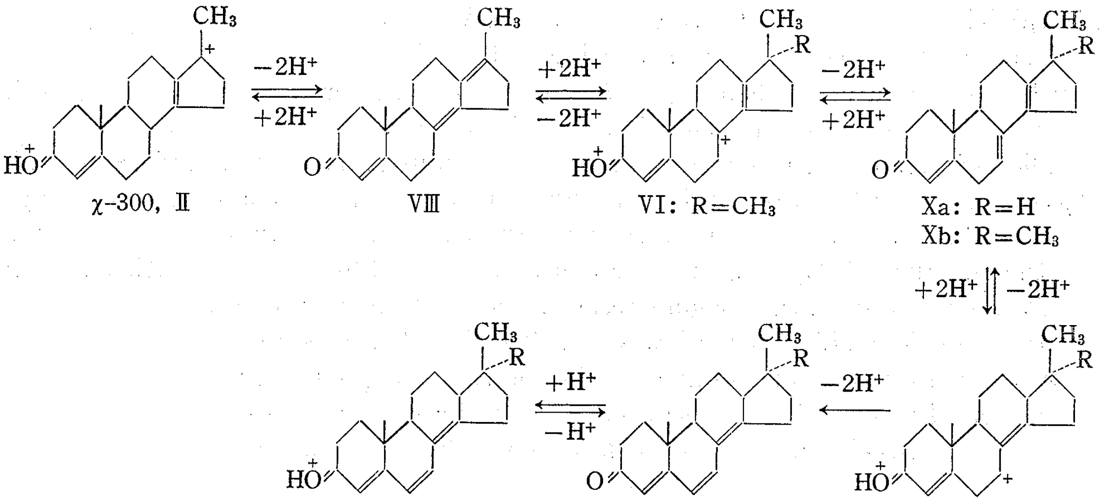


Fig. 4. Reversible Change between χ-484(XIIa) and its Conjugate Base (XIa)

————: CH_2Cl_2 solution of XIa (6 μg/ml).
– – – –: CH_2Cl_2 solution of XIa with one drop of 70% $HClO_4$.
—·—·—: CH_2Cl_2 layer removed $HClO_4$ by shaking with H_2O.
- - - - -: 65.0% H_2SO_4 solution of XIa (4 μg/ml).



χ-300, II
VIII
VI: R=CH₃
Xa: R=H
Xb: R=CH₃
χ-484, XIIa: R=H
XIIb: R=CH₃
XIa: R=H
XIb: R=CH₃

Chart 4. Mechanism of Formation of Chromophoric χ-484

The isooctane solution of XIa was shaken with sulfuric acids of various concentrations and the absorption intensity at 327 nm of the organic layer was measured and plotted against H_0 value of the sulfuric acid as shown in Fig. 5. Though XIa was not confirmed to be the Hammett's base, the pK_a value obtained by this method was −4.27.

Taking these findings into consideration, it may be concluded that the chromogenic reaction of testosterone (I) with sulfuric acid proceeded as shown in Charts 1 and 4. At the early stage of the reaction (Chart 1), high acidity as well as oxidative potentiality are necessary for dehydration and oxidation to form the χ-300(II). In the conversion of II to the chromophoric χ-484 (XIIa), the acid strength of the medium participates in the equilibria between II and its conjugate base (VIII) and also between XIIa and XIa. Since XIIa was formed from II *via* its conjugate base (VIII) by the acid-catalized isomerization, H_0 value of the acidic medium which shifts the former equilibrium to the side of VIII, is appropriate for the isomerization and its value may be above −3 corresponding to the pK_a value of II. On the contrary, the H_0 value should be below pK_a (−4.27) of XIIa in order to shift the latter equilibrium to the side of XIIa.

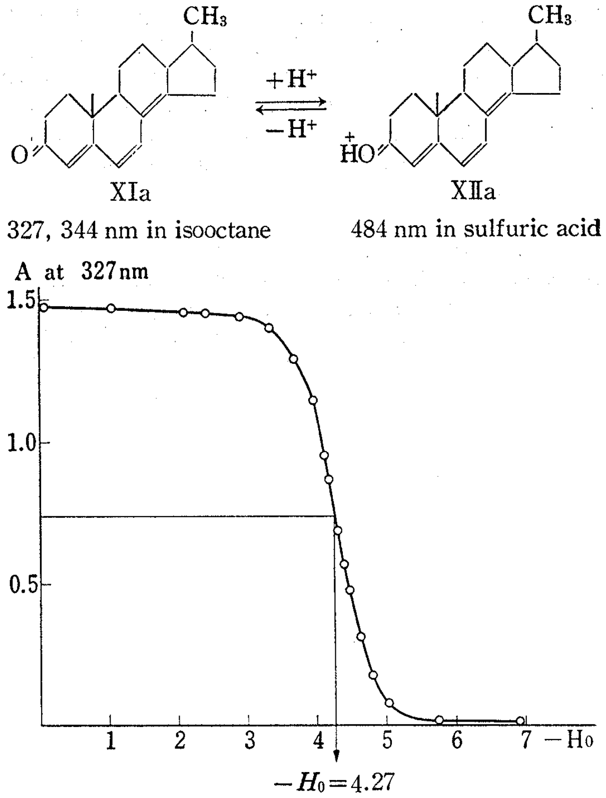


Fig. 5. Measurement of pK_a of χ-484(XIIa)

Absorbance at 327 nm of XIa (5.20×10^{-5}M in isooctane) was measured after shaking with same volume of H_2SO_4.

The chemical structure and the formation mechanism of another chromophoric species, χ-600, are now under investigation.

Experimental[6]

Material——A 1:1 mixture of 13ξ,17ξ-epoxy-17ξ-methyl-18-norandrost-4-en-3-one (III) and 13ξ,17ξ-epoxy-17ξ-methyl-18-norandrost-4-en-3-one (IV) was prepared as reported in the previous paper.[1] 17,17-Dimethyl-13ξ,14ξ-epoxy-18-norandrost-4-en-3-one (V) and 17β-methyl-18-norandrosta-4,6,8(14)-trien-3-one (XIa) were prepared by the methods of Torreilles[7] and Toth,[3h] respectively.

Reaction of V with Sulfuric Acid——A mixture of V (163 mg) and 62.0% H_2SO_4 (3 ml) was made homogeneous by vigorous shaking and allowed to stand at room temperature for 5 min. The mixture was dropped into ice-water (150 ml) under vigorous stirring and extracted with ether. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The oily residue obtained was submitted to column chromatography on silica gel (10 g, 70—230 mesh). Evaporation of the solvent from the fraction eluted by hexane–benzene (3:1) left a oily residue (85 mg) which was then submitted to a preparative TLC and developed twice by benzene–acetone (9:1). Elution of the adsorbent corresponding to the spot of *Rf*= 0.57 with benzene gave a slight yellow oil (64 mg). Crystallization of the oil was in no success but its spectral data were in good agreement with those[3e] of 17,17-dimethyl-18-norandrosta-4,6,8(14)-trien-3-one (XIb).

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6) Ultraviolet (UV) and visible (V) spectra were recorded on Hitachi Model ESP-3T spectrometer. NMR spectrum measurement were run on Hitachi Model R-20-B spectrometer at 60 MHz using tetramethylsilane as an internal standard. Abbreviation used s=singlet, d=doublet. Mass spectra (MS) were measured by Hitachi Model RMU-6R spectrometer. For preparative thin-layer chromatography (TLC) silica gel (Wakogel B-5F) was used as an adsorbent.

7) J. Torreilles and A.C. de Paulet, *Bull. Sci. Chim. France*, **1968**, 4886.

Anal. Calcd. for $C_{20}H_{26}O$: C, 85.05; H, 9.28. Found: C, 85.31; H, 9.16. MS *m/e*: 282 (M^+), 267 (M^+-CH_3). UV λ_{max}^{EtOH} nm (ε): 353 (24000), 286 (7800). IR ν_{max}^{Nujol} cm^{-1}: 1663, 1650 (shoulder) (C=O), 1590 (C=C). NMR (10% solution in $CDCl_3$) δ: 0.67 (3H, s, $C_{17}\beta$-CH_3), 1.00 (3H, s, $C_{17}\alpha$-CH_3), 1.10 (3H, s, C_{10}–CH_3), 5.73 (1H, s, C_4–H), 6.02 (1H, d, $J=9$ Hz, C_6–H), 6.63 (1H, d, $J=9$ Hz, C_7–H).

Absorption Spectra——Unless otherwise stated, 5 ml of H_2SO_4 was added to a dried sample at room temperature. The mixture was shaken vigorously to make a homogeneous solution and measured an absorbance at 25°.

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