[Chem. Pharm. Bull. 21(6)1205—1212(1973)]

UDC 547.92.04 : 546.137-33.04

Chromogenic Reactions of Steroids with Strong Acids. I. On the Reaction of Testosterone with Perchloric Acid[1]

Michiya Kimura and Kazuaki Harita

Faculty of Pharmaceutical Sciences, Hokkaido University[2]

(Received August 5, 1972)

From the 70% (w/w) $HClO_4$ solution saturated with testosterone (I) at room temperature, colorless needles, of 1 : 1 complex (II) of I and $HClO_4$ were isolated. When 0.2% (w/v) chloroform solution of I was heated with the equal volume of $HClO_4$ under nitrogen stream for 30 min at 60—65°, the steric isomers ($m/e=272$) of 17-methyl-18-norandrost-4-en-3-one (III) were obtained from the organic layer. The deep-green acid layer gave the mixture of 17-methyl-18-norandrosta-4,13(17)-dien-3-one (IV) and 17-methyl-18-norandrosta-4,13(14)-dien-3-one (V). Standard specimens of IV and V gave easily the similar absorption spectra to that (Fig. 1) due to I, when they were dissolved in the dichloromethane solution saturated with $HClO_4$. The dienones, IV and V, also gave the same spectra even in the moderately acidic conditions where I still remained inert. Partially hydrogenated product (III), on the contrary, failed to show any visible light absorption in the acid medium. It seems that the isomeric olefins, IV and V, are the intermediates in the present chromogenic reaction and the carbonium ions (VIII) formed by the protonation of them may play an essential part in producing the chromophore.

Various steroids are known to produce the chromophoric species when they come into contact with perchloric acid.[3] For the measurement of testosterone (17β-hydroxyandrost-4-en-3-one) (I), were employed various acids such as sulfuric acid, phosphoric acid, perchloric acid, and antimony trichloride. While extensive studies have thus been made on the colorimetric as well as fluorimetric measurements of various steroidal compounds, little is known on the detailed chemistry of their behavior in the acid medium. Formerly, the sequence of reactions was assumed that, when a steroidal mono-olefin is brought into contact with perchloric acid, the initial protonation leads to form the colorless sterolium perchlorate which is then dehydrated by excess acid to give a colorless diene with subsequent formation of the strongly colored halochromic salt.[4] On investigating the chromogenic reactions of steroids with strong acids, the following steroidal olefins have been assumed as the essential precursors of the corresponding chromophores: 17-methyl-3β-formyloxy-18-norandrost-13(17)-ene in the reaction of androstane-3β,17α-diol with formic acid,[5] 3,3′-bi(cholesta-2,4-diene)[6] or more highly conjugated polyolefinic monomer[7] in the Liebermann–Burchard color reaction[8] of cholesterol, the dimer cited above and cholesta-3,5-diene[9] in the color reaction of cholesterol with the mixture of perchloric acid, phosphoric acid, and ferric chloride, 17-methyl-3-methoxy-

1) Preliminary accounts of a part of this work have been published: M. Kimura, K. Harita, and T. Miura, *Chem. Pharm. Bull.* (Tokyo), **20**, 1829 (1972).
2) Location: *Nishi-6-chome, Kita-12-jo, Kita-ku, Sapporo, 060, Japan.*
3) *a*) H. Tauber, *Anal. Chem.*, **24**, 1494 (1952); *b*) D. Pontius, I. Beckmann, and K.D. Voigt, *Acta Endocrinol.* (Copenhagen), **20**, 19 (1955); *c*) W.D. Brown, *Austral. J. Exptl. Biol. Med. Sci.*, **37**, 523 (1959); *d*) S.C. Slack and W.J. Mader, *Anal. Chem.*, **33**, 625 (1961).
4) W. Lange, R.G. Folzenlogen, and D.G. Kolp, *J. Am. Chem. Soc.*, **71**, 1733 (1949).
5) K. Miescher and H. Kägi, *Helv. Chim. Acta*, **32**, 761 (1949).
6) T. Watanabe, *Eisei Shikenjo Hokoku* (*Bull. Natl. Inst. Hygien. Sci.*), **74**, 111 (1956); *idem, ibid.*, **77**, 81, 87 (1959).
7) C.H. Brieskorn and H. Hotmann, *Arch. Pharm.*, **292**, 485 (1959); *idem, ibid.*, **297**, 577 (1964).
8) C. Liebermann, *Ber.*, **18**, 1803 (1885); H. Burchard, *Chem. Zentr.*, **61**, I, 25 (1890).
9) T. Momose, Y. Ueda, M. Kageura, T. Masumura, and K. Ohta, *Chem. Pharm. Bull.* (Tokyo), **12**, 1415 (1964).

18-norestra-1,3,5(10),8(9)-tetraene as one of the possible isomeric species[10] in the Kober color reaction[11] of methylated estradiol (3-methoxyestra-1,3,5(10)-trien-17β-ol), and 17,17-dimethyl-18-norandrosta-4,6,8(14)-trien-3-one[12] in the reaction of 17-methyl-6-dehydrotestosterone (17α-methyl-17β-hydroxyandrosta-4,6-dien-3-one) with 62% sulfuric acid. This paper deals with structure elucidation of the main products and a plausible explanation of their formation, which were obtained from the chromogenic reaction of I with perchloric acid.

Since steroidal olefins are weak bases in their polarized states, they can form the salts such as sterolium perchlorate[4] with strong acid, which are decomposed into the original olefins by the more strong base such as water.[13] About ten-fold excess of 70% (w/w) perchloric acid was once able to dissolve I at 25° forming yellow solution which gave then colorless needles after allowing to stand for 30 minutes and gave also green crystals on diluting the filtrate with water. The former needles were proved to be 1:1 complex (II) consisting of I and perchloric acid. The latter crystals were soluble in dichloromethane as well as chloroform giving blue color and were proved to be composed of I and perchloric acid carrying minor amounts of unknown substances. When each of the residues, which were given from the refluxing mixtures of I and 70% perchloric acid in different non-polar solvents at their respective boiling points as described in the experimental part, was dissolved in the dichloromethane solution saturated in advance with this acid, similar absorption spectra were observed as shown in Fig. 1. On the basis of these facts, chloroform solution of I was heated with equal volume of 70% perchloric acid under nitrogen for 30 minutes at 60—65°. The product-analysis and structure-elucidation were then carried out on the organic as well as acid layers.

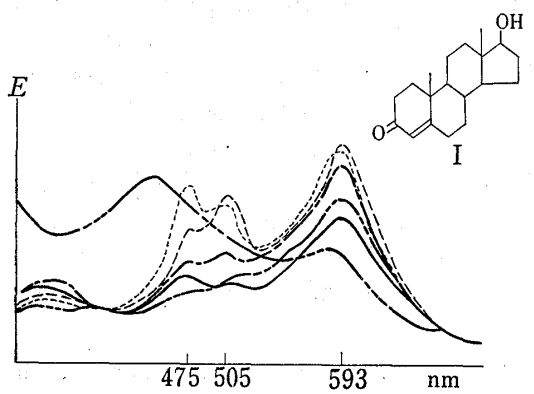

Fig. 1. Absorption Spectra in Dichloromethane Solution saturated with 70% Perchloric Acid

Taken on the residue from the reaction mixture of I and 70% $HClO_4$ in the organic solvent: CCl_4 (——), $CHCl_3$ (— — —), CH_2Cl_2 (- - - - -), C_6H_6 (— - —), $ClCH_2CH_2Cl$ (— — —), CH_3CN (— — — —).

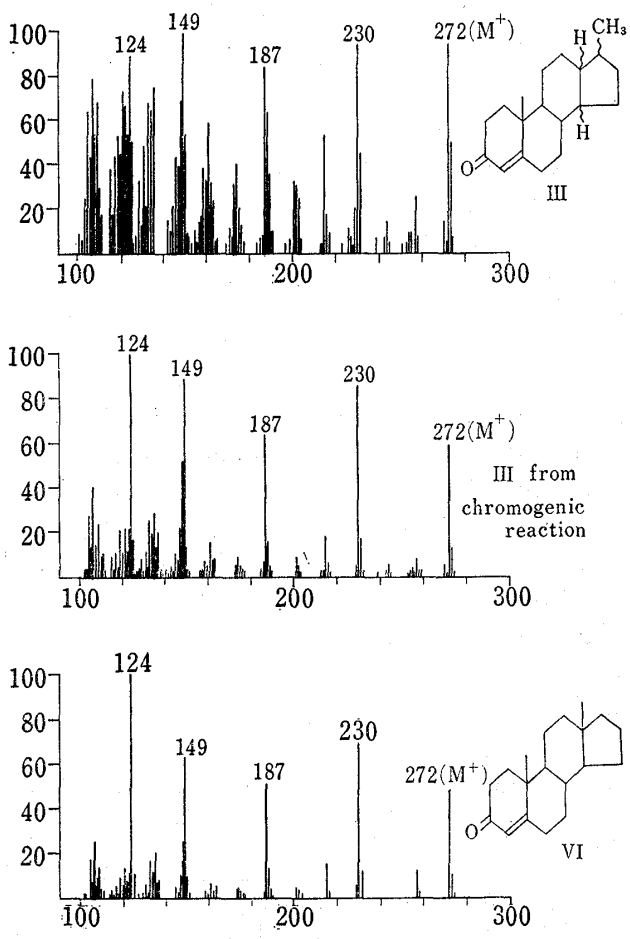

Fig. 2. Mass Spectra of III from Chromogenic Reaction and of Authentic III as well as VI

10) M. Kimura, K. Akiyama, K. Harita, T. Miura, and M. Kawata, *Tetrahedron Letters*, **1970**, 377.
11) S. Kober, *Biochem. Z.*, **239**, 209 (1931); *idem*, *Biochem. J.*, 32, 357 (1938).
12) W. Sadée, S. Riegelman, and L.F. Johnson, *Steroids*, **17**, 595 (1971).
13) H. Schaltegger, *Helv. Chim. Acta*, **29**, 285 (1946).

The organic layer was washed with water and evaporated. Chromatography of the residue on alumina gave the oily substance as a main constituent which was elucidated to be 17-methyl-18-norandrost-4-en-3-one (III) as follows. The synthetic specimen of III which was prepared from the mixture of 17-methyl-18-norandrosta-4,13(17)-dien-3-one (IV) and 17-methyl-18-norandrosta-4,13(14)-dien-3-one (V) and highly purified by column as well as preparative thin-layer chromatography (TLC) was, as might be expected, recognized to be the mixture of steric isomers through mass spectroscopy (MS) (Fig. 2) and the analysis by gas-liquid chromatography (GLC) giving double peaks, the retention times (t_R, min) of which were 5.5 and 6.3. Although the oily substance from the organic layer mentioned above gave single spot in TLC similarly to that of the synthetic specimen of III, GLC exhibited three peaks having t_R values of 5.5, 6.3, and 7.0. The semicarbazone, mp 210—213°, of this oil was treated with pyruvic acid to regenerate the original ketones which showed again three peaks in GLC and two of them were identical with those due to the synthetic specimen of III. It was unfortunate that the chemical shift due to C-17 methyl group in nuclear magnetic resonance (NMR) spectroscopy was not clear, possibly because of the isomeric mixture. None of them was noticed to be identical with the authentic specimen of androst-4-en-3-one (VI), t_R=6.0, in GLC of their admixture, though they gave the similar fragmentation patterns in MS (Fig. 2) to that of VI (M^+ 272). In GC-MS (Fig. 3), each of the three peaks, t_R=5.5, 6.3, and 7.0, showed the identical pattern of 272 (M^+), 230, 187, 149, and 124, suggesting that the three components were steric isomers difficult to be isolated individually.

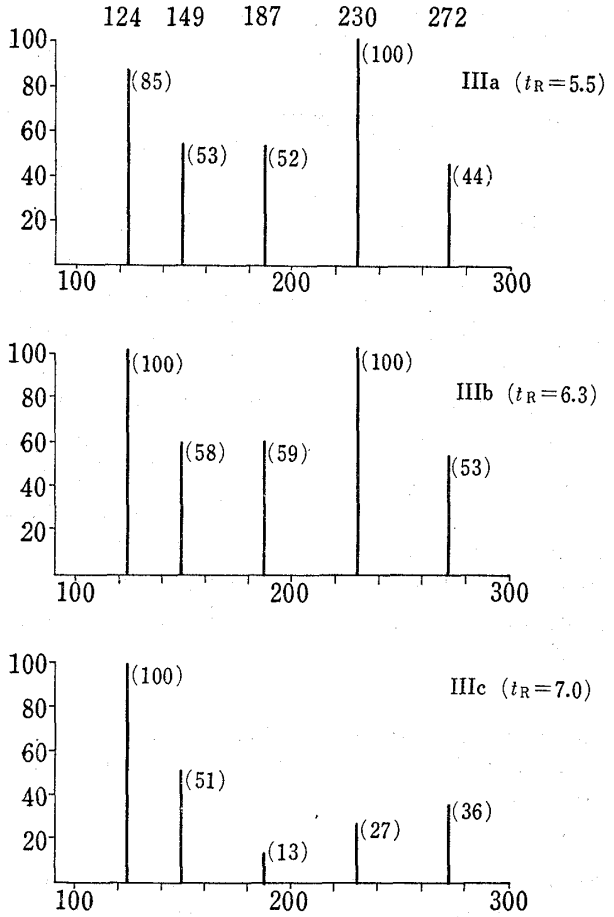

Fig. 3. Main Fragments in Mass Spectra of Gas Chromatographic Three due to Isomeric III

Numbers in parentheses reveal the relative abundances.

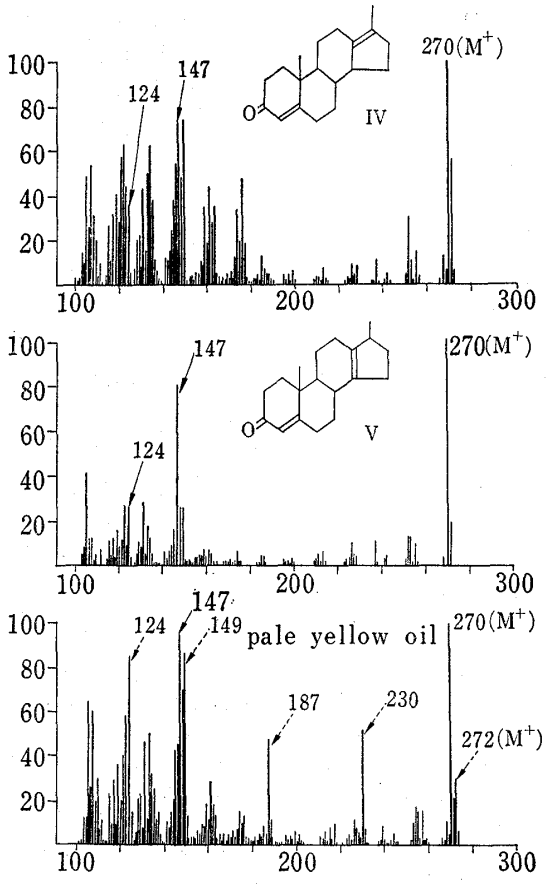

Fig. 4. Mass Spectra of IV, V, and the Pale Yellow Oil

The green colored layer of perchloric acid in the chromogenic reaction mixture mentioned above was diluted with water and extracted with chloroform. The pale yellow oil was obtained from the extract through column chromatography on alumina, which gave the signals of 4.25, 8.40, 8.84, 8.87, and 9.09 τ in NMR spectrum. The authentic specimen of IV, mp 110—111.5°, prepared by the method of Sondheimer, *et al.*[14] showed the signals of 4.25, 8.40, and 8.87 τ. The isomeric V, mp 103—111°, prepared by the method of Knox, *et al.*[15] gave the signals of 4.25, 8.84, and 9.09 τ in NMR. The signal of 4.25 τ may be due to the olefinic hydrogen at C-4 position. Knox, *et al.* reported that NMR of V showed the signals of 8.84 and 9.09 τ and assigned them to the methyl groups at C-19 and C-17, respectively. Although the formation of an alternative olefin, androsta-4,16-dien-3-one (VII) might have some possibility in the present reaction, the authentic specimen prepared by the method of Henbest, *et al.*[16] showed different chemical shifts of 8.78 and 9.18 τ due to the methyl groups at C-19 and C-18, respectively. GLC of the oily product revealed also two peaks, t_R=5.5 and 5.7, and each of the two isomers, IV and V, gave the same peak of t_R 5.7, contrary to 5.1 due to the other one (VII). Thus it seemed that the oily product consisted of two olefinic isomers, IV and V, carrying some amounts of impurity, t_R=5.5. As shown in Fig. 4, MS represented also the oily product as a mixture of these olefinic isomers and that III might be the impurity. Because of the poor yield of this isomeric mixture, isolation and recrystallization of the individual olefin were in no success, in spite of energetic purification.

Kägi and Miescher presented assumption on the mechanism of the color reaction of 17α-hydroxysteroids with acids.[17] They also reported the formation of 3β-formyloxy-φ-androstene (17-methyl-3β-formyloxy-18-norandrost-13(17)-ene) from 3β,17α-diformyloxyandrostane when it was heated at 100° in formic acid.[5] By refluxing epitestosterone (17α-hydroxyandrost-4-en-3-one) in formic acid for 50 minutes with resisting light under nitrogen stream, IV, V, as well as 17α-hydroxyandrost-4-en-3-one formate were obtained as expected.[18] The present authors obtained the isomeric mixture of 17-methyl-3-methoxy-18-norestra-1,3,5(10)-triene and the highly dehydrogenated 17-methyl-compounds such as 3′-methyl-7-methoxy-1,2-cyclopenteno-9,10-dihydrophenanthrene and its 6-sulfonate, when methyl ether of estradiol (3-methoxyestra-1,3,5(10)-trien-17β-ol) was heated with 78% (w/w) sulfuric acid for 20 minutes at 100°.[10] Sadée, *et al.* observed the migration of C-18 methyl to C-17 in the reactions of aldadienic acid (3-(3-oxo-17β-hydroxyandrosta-4,6-dien-17α-yl)propionic acid) and 6-dehydro-17α-methyltestosterone with 62% sulfuric acid, giving the corresponding 17β-methyl-18-nor-4,6,8(14)-trienones.[12] In 80 to 100% sulfuric acid, cyclo-aliphatic alcohols are dehydrated to their corresponding olefins which can then disproportionate to produce cycloalkenyl cations and saturated hydrocarbons.[19] In respect of the reaction mechanism of I with perchloric acid, therefore, it seems to be reasonable that the C-18 methyl group migrated to C-17 with the preceding retropinacolic elimination of C-17 hydroxyl group which is known to occur in acid medium.[20] Formation of the isomeric olefins, IV and V, may thus be reasonably clarified. The carbonium ions (VIII) once formed from these olefins in perchloric acid may then disproportionate to yield the allylic carbonium ions and the corresponding hydrogenated compound (III).

14) F. Sondheimer, O. Mancera, M. Urquiza, and G. Rosenkranz, *J. Am. Chem. Soc.*, **77**, 4145 (1955).
15) L.H. Knox, E. Velarde, S. Berger, D. Cuardriello, and A.D. Cross, *J. Org. Chem.*, **29**, 2187 (1964).
16) H.B. Henbest and W.R. Jackson, *J. Chem. Soc.*, **1962**, 954.
17) H. Kägi and K. Miescher, *Helv. Chim. Acta*, **22**, 683 (1939).
18) Details will be reported later.
19) G.A. Olah and C.U. Pittman, Jr., "Advances in Physical Organic Chemistry," ed. by V. Gold, Vol. 4, Academic Press, New York, 1966, p. 339; H.P. Leftin, "Carbonium Ions," ed. by G.A. Olah and P. von R. Schleyer, Interscience Publishers, New York, 1968, p. 353. N.C. Deno, "Carbonium Ions," in "Progress in Physical Organic Chemistry," ed. by S.G. Cohen, A. Streitwieser, Jr., and R.W. Taft, Jr., Vol. 2, Interscience Publishers, New York, 1964, p. 140.
20) W.F. Johns, *J. Org. Chem.*, 26, 4583 (1961); W.F. Johns and G.P. Mueller, *ibid.*, **28**, 1854 (1963).

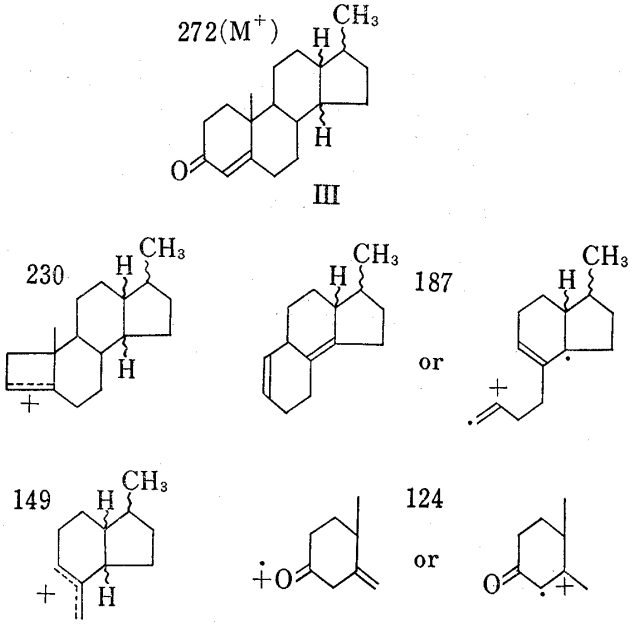

Chart 1

Structures of the main fragments from III were assigned by analogy of the results in the mass spectroscopic study on VI, reported by Shapiro and Djerassi.[21]

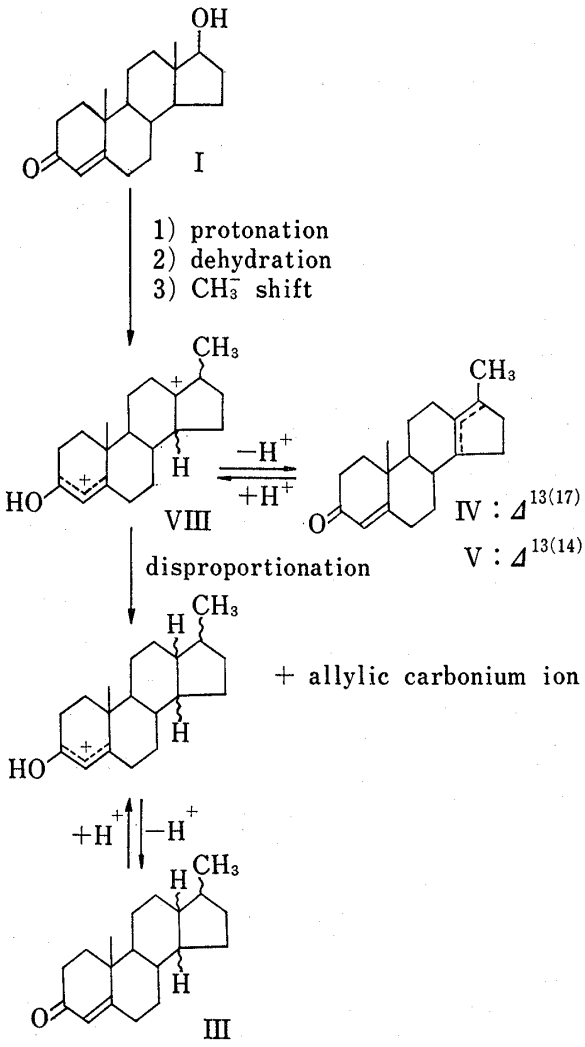

Chart 2

The color reaction of I with perchloric acid gave an absorption spectrum as shown in Fig. 1. Of three main products from this reaction, IV and V gave the similar spectra (λ_{max}= 475 and 593 nm) even in the moderately acidic conditions where I still remained inert. Another main product (III), on the contrary, failed to show any visible light absorption in this reaction. Recently, Jones, *et al.*[22] suggested that one of the Kober-chromophores[11] would be attributed to the resonating tertiary carbonium ions formed by the protonation of 17-methyl-3-hydroxy-18-norestra-1,3,5(10),13(17)-tetraene. In the preceding study,[10] the present authors proposed similar explanation about the Kober reaction of phenolic steroid on the basis of products analysis. This may be true in respect of the maximum light absorption at the wave length of 372 nm which appears in hot concentrated sulfuric acid at the earlier period of the Kober reaction.[23] However, no explanation is available at present for the more bathochromic absorptions which appear during elapse of reaction-time in the first phase and also in moderately concentrated sulfuric acid at the second phase of the Kober reaction. As might be assumed from the above mentioned spectroscopic phenomena giving the similar absorption maxima to those by I with perchloric acid, it seems that the isomeric 17-methyl-18-norsteroidal olefins, IV and V, are the intermediates in the present chromogenic reaction and the carbonium ions (VIII) formed by the subsequent protonation of them may play an essential part in producing chromophore. Discussions on the structure of the chemical species responsible for such characteristic light absorption are not appropriate until more evidence has been accumulated.

21) The conditions of J.F. Eastham and R. Teranishi, *Org. Syn.*, **35**, 39 (1955), were employed.
22) H.A. Jones and R. Hähnel, *Nature*, **215**, 1381 (1967); *idem*, *Steroids*, **13**, 693 (1969).
23) M. Kimura, K. Akiyama, and T. Miura, *Chem. Pharm. Bull.* (Tokyo), **20**, 2511 (1972).

Experimental[24]

1: 1 Complex (II) of I and Perchloric Acid——To 5 ml of 40% $HClO_4$ was dissolved 504 mg of I at 25° and the solution was allowed to stand for 30 min at the same temperature. Colorless needles formed were collected on a glass filter and dried in a vacuum desiccator overnight at 55°. The pale yellow needles thus obtained were very hygroscopic, soluble in acetone, insoluble in dichloromethane and chloroform, and decomposed into I with water. *Anal.* Calcd. for $C_{19}H_{28}O_2 \cdot HClO_4$: C, 58.76; H, 7.47; Cl, 9.02. Found: C, 56.58; H, 7.67; Cl, 10.51.

The filtrate through glass filter was diluted with water and the pale yellow needles precipitated were collected and dried overnight in vacuum desiccator at 55°. The crystals turned blue-green and was, in contrast to the 1:1 complex, soluble in dichloromethane promptly becoming blue (λ_{max}=482 and 596 nm). Neutralization of the acidic crystals with weak alkali gave I and minor amounts of the unknown colorless substances which were of, at least, four kinds and showed different *Rf*- as well as t_R-values from those of I in TLC and GLC, respectively.

Reactions of I with 70% Perchloric Acid in Organic Solvents (Fig. 1)——A stirred mixture of I (10 mg), 70% $HClO_4$ (5 ml) and solvent (5 ml) was refluxed for 30 min under N_2 stream. The solvents used and reaction temperatures were CCl_4, $CHCl_3$, CH_2Cl_2, C_6H_6, and $ClCH_2CH_2Cl$ and 77°, 62°, 40°, 80°, and 83°, respectively. The acid layer was diluted with ice-water, neutralized with 10% NaOH, extracted with $CHCl_3$, and dried over anhydrous Na_2SO_4. Evaporation of $CHCl_3$ *in vacuo* gave the residue, on which was taken the absorption spectrum in dichloromethane solution saturated with 70% $HClO_4$ as shown in Fig. 1. None of signals due to I was observed in the residue when it was submitted to TLC and GLC.

Formation and Isolation of Products from Reaction of I with 70% Perchloric Acid in Chloroform——A stirred mixture of I (40 g), 70% $HClO_4$ (400 ml), and $CHCl_3$ (400 ml) was heated at 60—65° for 30 min under N_2 stream. The dark-green acid layer was then diluted with ice-water and extracted with $CHCl_3$. The chloroform solution was washed with water, dried with anhydrous Na_2SO_4, and evaporated *in vacuo* giving red-brown oil A (25 g). As for the chloroform layer of the reaction mixture, it was washed with 10% NaOH, dried over anhydrous Na_2SO_4. Evaporation of chloroform *in vacuo* left brown-yellow oil B. When a stirred mixture of I (11 g), 70% $HClO_4$ (200 ml), and benzene (300 ml), in stead of $CHCl_3$ solvent, was heated at 70° for 70 min under N_2 and similarly worked up, 6.1 g of the oil B was yielded.

Isomeric Mixture of 17-Methyl-18-norandrost-4-en-3-one (III)——The brown-yellow oil B (6.1 g) was submitted to column chromatography on neutral alumina (300 g, 100—200 mesh) by eluting with *n*-hexane–benzene (45: 55). The first eluate (400 ml) was discarded and the fractions II—VII (7.5 l) were collected. Evaporation of solvent *in vacuo* left a colorless oil (4.8 g). Although TLC with different solvent systems gave single spot, GLC exhibited three peaks (t_R=5.5, 6.3, and 7.0). UV λ_{max} (EtOH): 240 nm (log ε=4.2), λ_{max} (CH_2Cl_2 saturated with 70% $HClO_4$): 288 nm. IR ν_{max} (direct): 1670, 1620 cm^{-1}. NMR ($CDCl_3$): τ 4.26 (s, 4-H), 8.85 (s, 19-H), 9.0—9.2 (17-CH_3). Mass Spectrum: 272 (M^+), 230, 187, 149, 124. GC–MS: 272 (M^+) from each of three peaks; semicarbazone: mp 210—213° (EtOH) and regenerated III by pyruvic acid giving three peaks of the same t_Rs.

Mixture of 17-Methyl-18-norandrosta-4,13(17)-dien-3-one (IV) and 17-Methyl-18-norandrosta-4,13(14)-dien-3-one (V)——The red-brown oil A (25 g) was submitted to column chromatography on neutral alumina (850 g; 100—200 mesh) by eluting successively with hexane, hexane–benzene (95: 5; 90: 10; 70: 30; 10: 90), benzene, benzene–$CHCl_3$ (80: 20; 50: 50; 20: 80), $CHCl_3$, $CHCl_3$–MeOH (90: 10; 70: 30; 50: 50; 30: 70), and MeOH. Evaporation of the solvent from the fraction eluted by benzene (2.0 l) left a residue (1.9 g) which was then submitted to chromatography on silica gel (190 g). The first fraction (28 mg) eluted by 2.2 l of $CHCl_3$ was discarded. Evaporation of chloroform from the next eluate (350 ml) *in vacuo* left a residue (309 mg). Preparative TLC (Kieselgel HF-254, AcOEt: benzene=1: 1) gave three fractions: pale yellow oil (*Rf* 0.7, 198 mg, blue fluorescence under UV-lamp of 2536 Å), pale yellow oil (*Rf* 0.6, 43 mg, reddish purple fluoresc.), and yellow solid (*Rf* 0.4, 27 mg, blue fluoresc.). Crystallization of the oil (*Rf* 0.7) and the isolation of IV as well as V were in no success. UV λ_{max} (EtOH): 240 nm, λ_{max} (dichloromethane saturated with 70% $HClO_4$): 475, 505, and 593 nm. GLC: t_R=5.7 (with the peak of t_R 5.5 due to III coexisted). NMR ($CDCl_3$): τ 4.25, 8.40, 8.84, 8.87, and 9.09. Mass Spectrum: 270 (M^+), 147, and 124 (with the signals due to III coexisted: 272, 230, 187, and 149).

24) Melting points were taken on a micro hot-stage apparatus and are uncorrected. Ultraviolet (UV) and infrared (IR) spectral measurements were run on Hitachi Model 3T recording and JASCO Model IR-S spectrometer, respectively. NMR spectra were obtained by Hitachi Model H-6013 spectrometer at 60 Mc. MS spectra were measured by Hitachi Model RMU-6E. TLC was carried out on silica gel (WACO-GEL B-5, B-5-F, or Kieselgel HF-254) plate by the solvent system of AcOEt–benzene (1: 1) or (2: 1). GLC was run on Shimadzu Model GC-4A gas chromatograph at 200° using a stainless steel column (3 m×3 mm i.d.) of 1.5% SE-30 on Shimalite W (60—80 mesh) with a N_2 flow of 40 ml/min and a hydrogen flame ionization detector.

Preparation of Reference Substances——17-Methyl-18-norandrosta-4,13(14)-dien-3-one (V)[15]: A mixture of testosterone tosylate (10 g), CH_3COOK (10 g), and glacial acetic acid (200 ml) was refluxed for 24 hr under N_2. The crude products were then purified by column chromatography, preparative TLC and recrystallization from *n*-pentane-ether to give colorless needles, mp 103—111° (lit.,[15] mp 110—112°). UV λ_{max}^{EtOH}: 242 nm. IR ν_{max}^{Nujol} cm^{-1}: 1675. Mass Spectrum: 270 (M^+). NMR ($CDCl_3$) τ: 4.25 (s, C_4-H), 8.84 (s, C_{19}-H), 9.09 (C_{17}-CH_3). GLC: t_R=5.7; unstable for light and air exposure.

17-Methyl-18-norandrosta-4,13(17)-dien-3-one (IV)[14]: The mother solution from the recrystallization of V was evaporated *in vacuo* to leave a residue which was purified through column chromatography on silica gel as well as preparative TLC and was recrystallized repeatedly from ether-*n*-pentane to give colorless needles, mp 110—111.5° (lit.,[14] mp 113—114°). *Anal.* Calcd. for $C_{19}H_{26}O$: C, 84.39; H, 9.69. Found: C, 84.24; H, 9.66; $[\alpha]_D^{21°}$ ($CHCl_3$): +87° (c=1.57) (lit.,[14] +84°); Mass Spectrum *m/e*: 270 (M^+); NMR ($CDCl_3$) τ: 4.25 (s, C_4-H), 8.40 (s, C_{17}-CH_3), 8.87 (s, C_{19}-H); GLC: t_R=5.7; unstable for light and air exposure.

Androsta-4,16-dien-3-one (VII)[16]: After a mixture of testosterone tosylate (0.51 g), *n*-Bu_4NOAc (2.27 g), N-methyl-2-pyrrolidone (10 ml) was heated at 160° for 8 hr under N_2, it was evaporated to dryness. The residue was worked-up as usual, then purified by column chromatography, and finally recrystallized from ether–hexane to colorless needles, mp 133—134° (lit.,[16] mp 131—133°), 32% yield. UV λ_{max}^{EtOH}: 240 nm; IR ν_{max}^{Nujol} cm^{-1}: 1675, 1620; NMR ($CDCl_3$) τ: 9.17 (C_{18}-CH_3), 8.78 (C_{19}-H), 4.25 (C_4, C_{16}, C_{17}-H); GLC: t_R=5.1.

Androsta-4-en-3-one (VI)[25]: A mixture of dehydroepiandrosterone (3β-hydroxyandrosta-5-en-17-one, 1.71 g), 90% hydrazine hydrate (0.85 ml), KOH (1.02 g), and triethylene glycol (11.9 ml) was refluxed at 142—146° for 24 hr under N_2 and was then dehydrated by heating for another 1.25 hr without condenser. After refluxing further at 201—211° for 4 hr, ice–water was added to the reaction mixture followed by extracting with ether. Organic layer was worked-up as usual and the extracts were recrystallized from acetone to give colorless needles of androsta-5-en-3β-ol, mp 136.5—138.5° (lit.,[25] mp 133—134°). *Anal.* Calcd. for $C_{19}H_{30}O$: C, 83.15; H, 11.02. Found: C, 83.25; H, 11.27. Mass Spectrum *m/e*: 274 (M^+). A portion (1.3 g) of the alcohol thus obtained was converted to the ketone (VI) by the Oppenauer oxidation.[21] The crude products were then purified by column chromatography and finally recrystallized from EtOH to give colorless needles, mp 104.5—105.5° (lit.,[25] mp 104—105°); Semicarbazone: mp 209—211° (EtOH); UV λ_{max}^{EtOH}: 242.5 nm (lit.,[25] 240 nm, log ε=4.23); Mass Spectrum *m/e*: 272 (M^+), 230, 187, 149, 124. *Anal.* Calcd. for $C_{19}H_{28}O$: C, 83.77; H, 10.36. Found: C, 83.55; H, 10.31; GLC: t_R=6.0.

17-Methyl-18-norandrost-4-en-3-one (III): In a mixture of AcOH (12 ml), AcOEt (8 ml), and PtO_2 (510 mg), a mixture (720 mg) of IV and V was hydrogenated at 22° for 9 hr. The yellow solution with red fluorescence turned colorless. The reaction mixture was then filtered and the precipitate remained were washed with AcOEt. Evaporation *in vacuo* of solvent from the filtrate combined with AcOEt washings left a residue which was dissolved in benzene, and washed successively with water, aqueous $NaHCO_3$, and water. After usual work-up, yellow oil (686 mg) was obtained from the benzene solution. In order to hydrolyze the acetate formed, a mixture of the oil (680 mg), KOH (0.5 g), MeOH (20 ml), and water (0.5 ml) was refluxed for 2 hr. After evaporating MeOH and extracting the remained aqueous layer with benzene, the organic layer was then worked-up as usual. Evaporation of solvent from the benzene solution left yellow oil (crude 17-methyl-18-norandrostan-3-ol, 610 mg). To a stirred acetone solution (5 ml) of this oil, was added dropwise a mixture of CrO_3 (0.2 g), conc. H_2SO_4 (0.2 ml), and water (1 ml) at 0° for 5 min. The reaction mixture was combined with aqueous solution (100 ml) saturated with NaCl and extracted with benzene. The organic layer was washed successively with aqueous NaCl, 5% $NaHCO_3$, water, and dried over anhydrous Na_2SO_4. Evaporation of solvent from the benzene solution left yellow oil (crude 17-methyl-18-norandrostan-3-one, 570 mg), Mass Spectrum *m/e*: 274 (M^+). To a stirred DMF solution (15 ml) of this oil (558 mg) and *p*-toluenesulfonic acid (33 mg), was added drpwise DMF solution (2 ml) of Br_2 (0.5 g) for 45 min at room temperature and the reaction mixture was stirred further for 1 hr, to which was then added ether (200 ml). Ether solution was washed successively with water, aqueous $NaHCO_3$, water and dried over anhydrous Na_2SO_4. Evaporation of ether left yellow resinous residue (crude 4-bromo-17-methyl-18-norandrostan-3-one, 766 mg). A mixture of this residue (766 mg), DMF (15 ml), and LiCl (316 mg) was heated at 100° for 2 hr under N_2. After adding water, the mixture was extracted with ether. The organic layer was then washed with dil. HCl and water and dried over anhydrous Na_2SO_4. Evaporation of ether left a brown oil (556 mg) which was submitted to chromatography on alumina (25 g, 100—200 mesh) by eluting successively with hexane (100 ml), hexane–benzene (90: 10, 150 ml; 70: 30, 50 ml; 50: 50, 50 ml), benzene (300 ml), and MeOH (50 ml). Evaporation of solvent from the fraction eluted with the last 300 ml of hexane–benzene (50: 50) left a residue (69 mg) which was then submitted to preparative TLC on Kieselgel HF-254 by eluting with AcOEt–benzene (1: 1). The spot area of *Rf* 0.6 was scratched and extracted with AcOEt. Evaporation of solvent left a colorless oil. GLC: t_R=5.5, 6.3, and 7.0; semicarbazone: mp 210—213° (EtOH). To an acetic acid solution (1.5 ml) of the semicarbazone (0.04 g), was added pyruvic acid (0.2 ml) and water (0.5 ml) and the mixture was heated at 60° for 22 hr. After extracting the mixture

25) R.H. Shapiro and C. Djerassi, *J. Am. Chem. Soc.*, **86**, 2825 (1964); A. Butenandt, L.K.-Poschmann, G. Failer, U. Schiedt, and E. Bickert, *Ann.*, **575**, 123 (1952).

with $CHCl_3$, the organic layer was washed with alkali and water and then dried over anhydrous Na_2SO_4. Evaporation of $CHCl_3$ left a residue which was submitted to preparative TLC. The spot area of *Rf* 0.6 was scratched and worked up as usual to give an oil (III). GLC: $t_R = 5.5$ and 6.3. UV $\lambda_{max}^{CH_2Cl_2}$: 241 nm, λ_{max} (CH_2Cl_2 saturated with 70% $HClO_4$): 290 nm (no absorption on the visible region). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1618, 1660 (C=C–CO). Mass Spectrum *m/e*: 272 (M^+), 230, 187, 149, 124. NMR ($CDCl_3$) τ: 4.26 (s, C_4-H), 8.85 (s, C_{19}-H), 9.02 (C_{17}-CH_3).

Acknowledgement The authors are gratefully acknowledged to Shimadzu Seisakusho Co. for the measurement of GC-MS. Thanks are also due to the staffs of the central analytical laboratory of this Faculty for the elemental analysis and measurements of NMR and MS spectroscopies.