methanol to give 55% yield of the ketal epoxide (**2**), mp 153—155°, NMR: 3.30 (15α-H).[11] Reduction of **2** with the highest grade lithium aluminum hydride in refluxing ether provided 14β-hydroxy compound in 80% yield, which was subjected to the hydrolysis of the ethylene ketal group under carefully controlled condition (in methanol containing 1/5 volume of 50% acetic acid, 65°, 30 min) to give 85% yield of 3β,14β-dihydroxy-5α-pregnan-20-one (**3**), mp 240—244°. IR ν_{max} (KBr): 3520, 3400 (OH), 1695 cm^{-1} (20-one). NMR: 0.80 (19-H), 0.95 (18-H), 2.24 (21-H), 3.60 (3-H). Mass Spectrum *m/e*: 334.252 (M^+, Calcd., 334.251), 316 (M^+-H_2O), 306 (M^+-C_2H_4), 248 (306-aceton), 230 (248-H_2O), 215 (230-Me). ORD (dioxan) $[\alpha]^{25}$(nm): +806° (308) (peak), −431° (270) (trough).

The second route which does not involve the protection of 20-ketone group was investigated on 3β-acetoxypregn-5,14-dien-20-one (**4**). Treatment of **4** with 1.2 equivalents of N-bromoacetamide in acetone-acetic acid at 0° for 1 hr afforded very unstable bromohydrin (**5**). It was immediately subjected to hydrogenolysis by hydrogen saturated Raney Ni in methanol at pH=6.7 (adjusted by an acetate buffer). Chromatography of the reduction product on deactivated neutral alumina gave three products (in the order of elution): **4** (—30%), 3β-acetoxy-14β,15β-epoxypregn-5-en-20-one,[12] and 3β,14β-dihydroxypregn-5-en-20-one 3-acetate (**6**) (—35%), mp 192—195°. The structure and the stereochemistry of **6** was confirmed by the following spectroscopic data: IR ν_{max} (KBr): 3360 (OH), 1735 (AcO), 1685 cm^{-1} (20-one). NMR: 0.98 (18- and 19-H), 2.05 (AcO), 2.25 (21-H), 2.85—3.1 (17α-H), 5.45 (6-H). ORD (dioxan) $[\alpha]^{25}$ (nm): +550° (308) (peak), −430° (269) (trough).

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12) Ref. 2, footnote 8).

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Chromogenic Reactions of Testosterone with Strong Acids

Despite numerous reports in the literature on the colorimetric determination of testosterone (I) with strong acids, the detailed chemistry in the reaction mixture is not fully understood. This communication deals with the products and the assignment of the chemical species responsible for the absorption maxima in the chromogenic reactions of I with perchloric acid as well as sulphuric acid.

Chloroform or benzene solution (400 ml) of I (40 g) was heated with the same volume of 70% (w/w) perchloric acid under nitrogen stream for 30 min at 60—65°. The residue of the organic layer was submitted to chromatography on alumina and the epimeric mixture of 17-methyl-18-norandrost-4-ene-3-one (II), UV $\lambda_{max}^{CH_2Cl_2}$: 241 nm, IR $\nu_{max}^{CHCl_3}$: 1618, 1660

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cm^{-1}, NMR (δ in $CDCl_3$): 5.74 ppm, Mass Spectrum *m/e*: 272 (M^+), 230, 187, 149, 124, was obtained as a main constituent. The colored layer of perchloric acid was diluted with water and extracted with chloroform. The products, 17-methyl-18-norandrosta-4,13(17)-diene-3-one[1] (III), mp 110—111.5°, *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), NMR (δ in $CDCl_3$): 1.13, 1.60 ppm, Mass Spectrum *m/e*: 270 (M^+), and 17-methyl-18-norandrosta-4,13(14)-diene-3-one[2] (IV), mp 103—111°, NMR (δ in $CDCl_3$): 0.91, 1.04, 1.16, 1.60 (due to III as an impurity) ppm, Mass Spectrum *m/e*: 270 (M^+), were obtained from this layer through column chromatography on alumina. After the mixture of I (336 mg), ethanol (2 ml), and 97.2% (w/w) sulphuric acid (0.5 ml) was kept at room temperature for 8 min, it was poured into ice-water and extracted with ethyl acetate. Ultraviolet spectrum (λ_{max}=350 nm) and gas liquid chromatography of the extracts revealed the presence of 17-methyl-18-norandrosta-4,6,8(14)-triene-3-one (V) in addition to relatively large amount of II. Another spot in thin-layer chromatography was found to be due possibly to 17-methyl-18-norandrosta-4,7,13(14)-triene-3-one (VI) or the olefinic isomer(s) on the basis of absorption (λ_{max}^{EtOH}: 242 nm) and mass (M^+: 268) spectra.

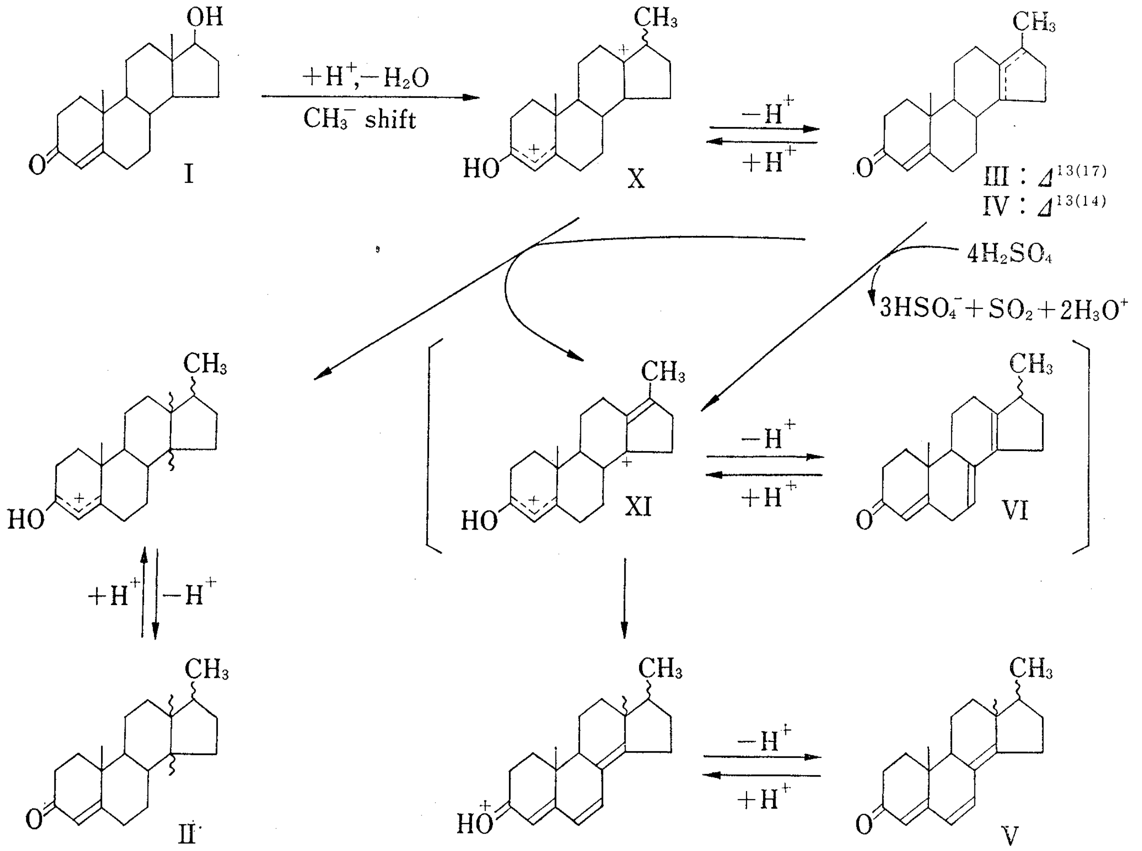

Chart 1

The absorption spectra (λ_{max}: 253, 298, 480, and 596 nm) of III and IV in cold and moderately concentrated sulphuric acid were almost identical with that given by the ordinary colorimetric procedures of I with the hot and concentrated acid. The olefins, III and IV, were, therefore, likely to be the essential intermediary chromogens. No absorptions in the visible region, on the other hand, were observed on I (λ_{max}=298 nm), II (295 nm), III (298 nm), IV (298 nm), androst-4-ene-3-one[3] (VII) (295 nm), and 5α-androstane-17β-ol[4] (VIII) (310 nm)

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in cold conc. sulphuric acid (Fig. 1). The maximum at 253 nm may chiefly be contributed by the α,β-unsaturated ketone structure. The absorptions at 295 nm have proved to be due to the hydroxyalkenyl carbonium ions (IX) formed from the α,β-unsaturated ketones.[5,6] Leftin[7] described that the 3000 (±50) Å bands which were shown by some hydrocarbons, alcohols, and olefins in sulphuric acid have assigned to the alkenyl carbonium ions formed. It seemed that the maximum absorptions at 298 nm shown by I, III as well as IV are doubly contributed by the protonated resonance hybrid (IX) and by the alkenyl carbonium ions as well. Deno, *et al.*[8] assigned the nuclear magnetic resonance (NMR) signals at 2.5—3.0 ppm to the methyl, methylene, and methin groups situated at the α-positions of alkenyl carbonium ions carrying deshielding effect. The NMR spectra of I shown in Fig. 2 may suggest the migration of 18-methyl group to give such carbonium ions in conc. sulphuric acid. The bands due to the alkenyl carbonium ions are also known to be intensified by some oxidizing agent such as selenic acid.[7] It was interesting in this respect that spectra of the ketonic olefins, III and IV, in the mixture of sulphuric and selenic acids showed: 1) the increase in absorptions at 298 nm with increase in the concentration of selenic acid and 2) the decrease in maximum absorptions at 253 as well as 298 nm with increase in those at the bathochromic regions, 480 and 596 nm, during elapse of reaction-time (Fig. 3). These results seemed to be best interpreted to indicate that III and IV are oxidized by conc. sulphuric acid (Fig. 1) or by selenic acid to give a series of polyolefinic ketones responsible for the absorptions at 298 nm as well as more bathochromic regions. In fact, the synthetic specimen of V, mp 159—164°, *Anal.* Calcd. for $C_{19}H_{24}O$: C, 85.02; H, 9.01, Found: C, 84.44; H, 8.98, UV $\lambda_{\max}^{\mathrm{EtOH}}$: 350 nm ($\varepsilon$ 28200), IR $\nu_{\max}^{\mathrm{Nujol}}$: 1653, 1640, 1585 cm^{-1}, NMR (δ in $CDCl_3$): 0.99, 1.06, 5.75, 6.04, 6.63, Mass Spectrum *m/e*: 268(M$^+$), gave the maximum at 480 nm in sulphuric acid and the intact trienone substrate (V) could almost quantitatively be recovered from the neutralized solution, as has been observed in the similar case of 17,17-dimethyl-18-norandrosta-4,6,8(14)-triene-3-one.[9]

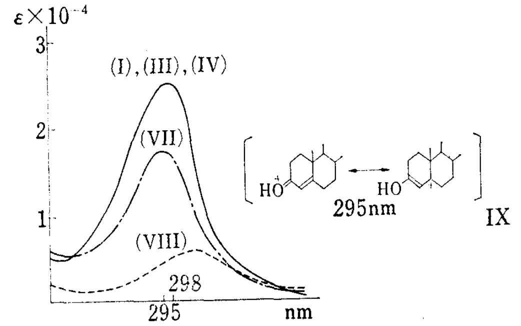

Fig. 1. Absorption Spectra in Sulphuric Acid

substrate: 150 μg 97.2% H_2SO_4: 10 ml at room temperature

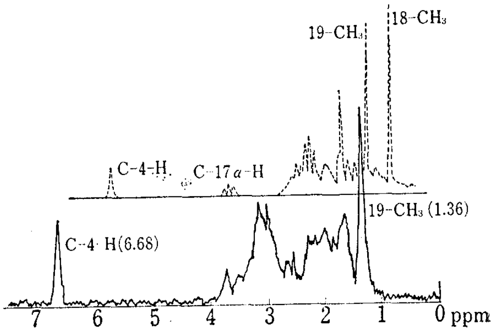

Fig. 2. NMR Spectra of Testosterone

testosterone: 26 mg
——: in 0.7 ml of conc. D_2SO_4 ------: in $CDCl_3$
external standard: 10% TMS-$CDCl_3$

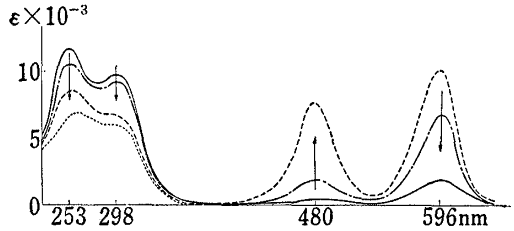

Fig. 3. Absorption Spectra of Dienones (III and IV) in Sulphuric Acid containing Selenic Acid

mixture of III and IV: 75 μg
0.005% H_2SeO_4–40%(v/v) H_2SO_4: 5 ml
——: 10 min ---: 2 hr ---: 74 hr ......: given by androst-4-en-3-one (VII)

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In respect of the reaction mechanism of I with strong acid, the 18-methyl group migrated to 17-carbon with the preceding retropinacolic elimination of 17-hydroxyl group to give the carbonium ion (X) which may then be dehydrogenated into the protonated states (XI) of trienone (VI) through disproportionation. The dienones, III and IV, which are the conjugate bases of X, may also be oxidized to VI by the acid. The isomerization of VI may subsequently occur in the acid medium to the conjugated trienone (V) responsible for the absorption maximum at 480 nm.

Details of the chemical species responsible for the light absorption at 596 nm are now under investigation.

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Syntheses of Isoalloxazines. A New Synthesis of Riboflavin

The customary synthetic routes to isoalloxazines involve the condensation of (a) *o*-phenylenediamines with alloxan, alloxantin, dialuric acid, halobarbituric acid or violuric acid, (b) 2-arylazoanilines with barbituric acid, (c) anilines with violuric acid, (d) *o*-benzoquinones with 5,6-diaminopyrimidines, (e) dimeric biacetyl and diaminouracils, or diacetyl and preformed lumazines, and (f) quinoxalines with guanidine. Recently, these procedures were well documented in the literature.[1] In continuation of our studies[2–14] on the syntheses of purine, pteridines and related systems, we are now reporting two new synthetic approaches to isoalloxazines.

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