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A Phosphorylation of Steroids and a Dienone-Phenol Rearrangement leading to a Secosteroidal Aldehyde Which has a Strong Toxicity

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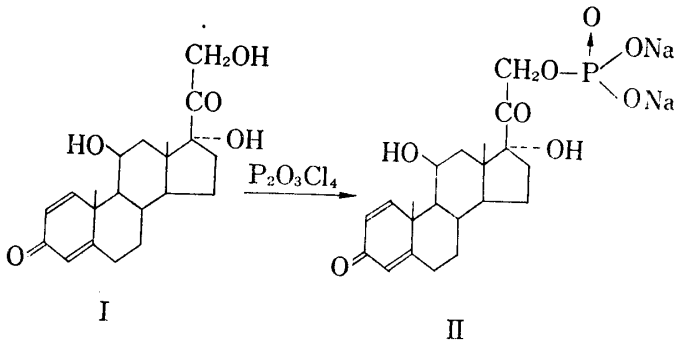
By the use of pyrophosphoryl chloride, 21-hydroxy-20-oxosteroids were phosphorylated in one step in quantitative yield. When the reaction was carried out on prednisolone at a higher temperature, a dienonephenol rearrangement and a simultaneous pinacolic shift took place resulting in the formation of 3,21-diphosphate of C-nor-9,10-secosteroidal aldehyde (A) as a by-product. This compound showed a cardioinhibitory and vasoconstricting activity and a strong toxicity to rabbits.

During the course of study on the synthesis of prednisolone phosphate, a cardioinhibitory and vasoconstricting substance has been isolated and found to be a rearrangement product of prednisolone. This report describes the chemistry of the new substance and some findings in animal experiments.

Wendler and his colleagues[2] developed an exceedingly simple procedure for the synthesis of triamcinolone acetonide 21-phosphates. They used an excess amount of pyrophosphoryl chloride based on Grünze's observation[3] to obtain the steroid phosphate in one step in essentially quantitative yield. It was stated in their patent application that the hydroxyl group at C-17 should be blocked by the formation of an ether and that the hydroxyl group at C-11 should be protected from the elimination reaction by the halogen group at C-9.

We independently applied the same reagent to 21-hydroxy-20-oxosteroids *e.g.* prednisolone (I) and dexamethasone (XXIII) with the unprotected hydroxyl groups and obtained their phosphates in more than 80% yields.[4] Our process consists of treating prednisolone (I), for example, with pyrophosphoryl chloride in a mixture of *m*-cresol and tetrahydrofuran at −40°, quenching the reaction mixture with ice water, adsorbing the phosphate on activated charcoal, washing with water to remove the inorganic phosphate and chloride, followed by elution of prednisolone phosphate (II) with a methanolic alkali solution. The effectiveness of this process may be understandable in view of the fact that reagents such as $POCl_3$ or PCl_5

1) Location: *Juso, Higashiyodogawa-ku, Osaka.*
2) H.L. Slates, S. Weber, and N.L. Wendler, U.S. Patent 3487077 (1965) [*Chemistry & Industry*, **1967**, 1174].
3) H. Grünze and W. Koransky, *Angew. Chem.*, 71, 407 (1959).
4) T. Miki and H. Masuya, J. Patent 597159 (1965).



I II

Chart 1

without base did not work on the 21-hydroxyl group at all and even though phospholyration occurred in the presence of base the main product was the disteroid phosphate.

When the crude prednisolone phosphate obtained by this reaction at the room temperature was intravenously injected into a rabbit, the animal exhibited a chronic convulsion, followed by respiratory depression or paralysis, and death occurred within 15 minutes. The sensitive method to detect the toxic activity was by its effect on isolated guinea pig auricle, where suppression of the contraction amplitude was observed (Fig. 1).

Chemistry of the Toxic Substance

Because the inhibitory action on the guinea pig auricle and the acute lethal doses in the rabbit were found proportional, chemical investigation was directed to the isolation of the toxic substance, using the inhibitory action as the index for tracing. It was found that a reaction at a higher temperature for a longer period seemed to give a product of stronger toxicity (Table I).

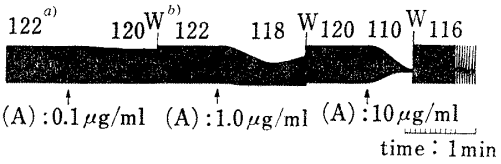


Fig. 1. Effect of Compound (A) on Isolated Guinea Pig Auricle

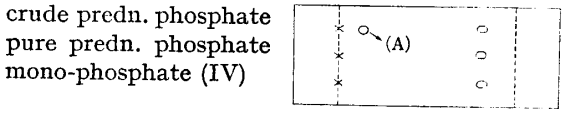


Fig. 2. TLC (PrOH: 28% aq. NH_4OH=1: 1)

TABLE I. Comparison with Toxicity (*in Vivo* and *in Vitro*) of Various Materials

Material	LD_{50} a) (Rabbit, *i.v.*)	IC_{50} b) (Guinea pig auricle)
Crude prednisolone phosphate	34.2 mg/kg	12.1 μg/ml
Purified toxic compound	2.24	0.7
Prednisolone phosphate	360	500

a) Male albino rabbits weighing 2—3 kg were used with observation period of 24 hr.
b) The 50% inhibited concentration of test materials in the final reaction solution.

Thin-layer chromatography (TLC) of the product and spraying of conc. sulfuric acid revealed a faintly red fluorescent spot ((A) in Fig. 2) in addition to a yellow fluorescent spot of prednisolone 21-phosphate (II). Water extracts of the former spot on a preparative thin-layer chromatogram showed a strikingly toxic effect on the guinea pig auricle. The extract was subjected to hydrolysis in a malt enzyme solution to precipitate a crystalline substance (V) which melted at 93°. On the other hand, when prednisolone 21-acetate (III) was treated with pyrophosphoryl chloride for several hours, a new monophosphate (IV) was obtained in a good yield. This was hydrolyzed in the malt enzyme solution to give the same substance as that obtained from the preparative thin-layer chromatogram. In this way the preparation of this compound for structure determination became within the range of possibility.

In Fig. 3, three aromatic protons and an aldehyde group are observed instead of the absorptions characteristic of the dienone system present in prednisolone. It reduced Fehling's solution, and was positive to the silver mirror test. The nuclear magnetic resonance (NMR) shows the presence of the 21-CH_2 group as in prednisolone and the colour reaction with bluetetrazolium was positive, suggesting that the dihydroxyacetone group at C-17 had undergone no alteration. From these facts, a sequence of rearrangements, as shown in Chart 2, was assumed to have taken place.

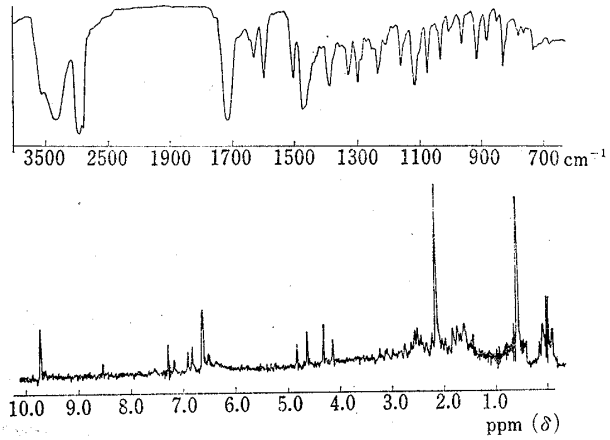


Fig. 3. IR and NMR Spectra of V

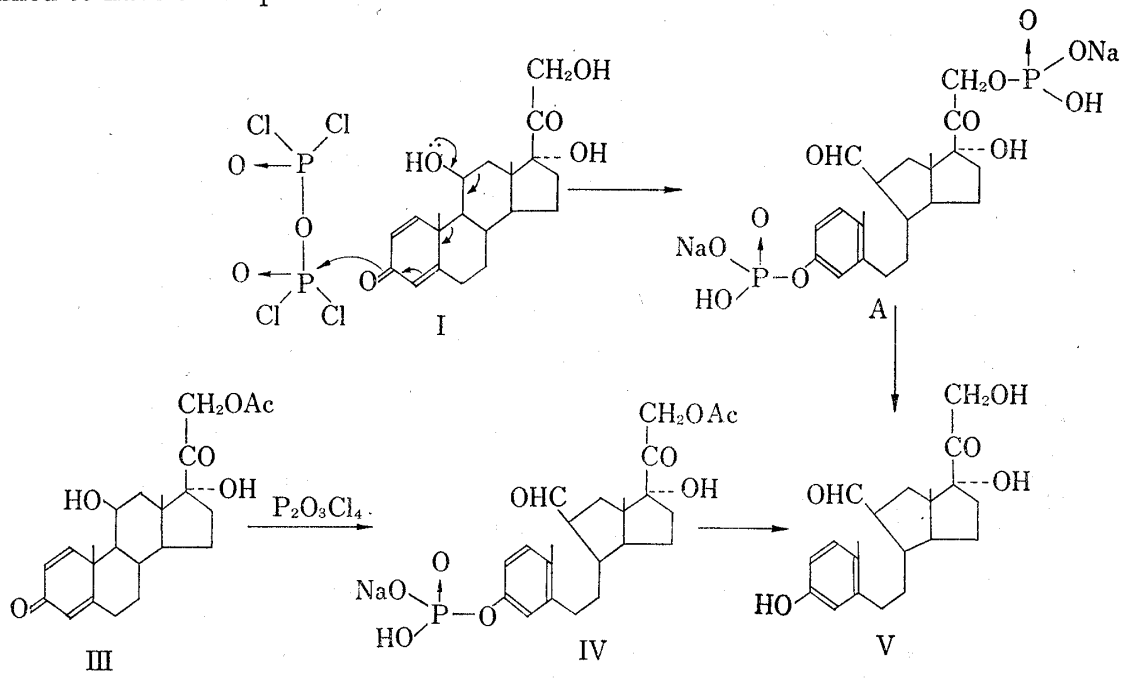


Chart 2

In order to prove this assumption and to determine the structure of the hydrolyzed product (V), a series of reactions, as shown in Chart 3, was carried out. Lithium aluminumhydride reduction and methylation with dimethyl sulfate followed by sodium periodate cleavage transformed the product (V) to a crystalline material (VII) of mp 85—86°, the infrared (IR) spectrum of which showed an absorption of 5-membered ring ketone at 1743 cm^{-1}. This absorption disappeared on sodium borohydride reduction in methanol. Chromic anhydride oxidation of VII or VIII afforded a ketocarboxylic acid (IXa) as an oily material and the IR spectrum displayed carbonyl bands at 1700 cm^{-1} and 1735 cm^{-1}. The ester (IXb) obtained by treatment with diazomethane revealed an additional methyl signal in NMR spectrum showing IXa to be a monobasic acid.

The above results were confirmed by another series of reactions, namely, methylation of V with methyl iodide in the presence of potassium carbonate, acetylation, chromic anhydride oxidation of the aldehyde (XI) to a carboxylic acid (XII), hydrolysis of the acetate and, finally, cleavage of the side chain with sodium bismuthate, giving the same ketocarboxylic acid (IXa). Thus it is considered that the D-ring is 5-membered and the dihydroxyacetone group also exists unchanged as in prednisolone.

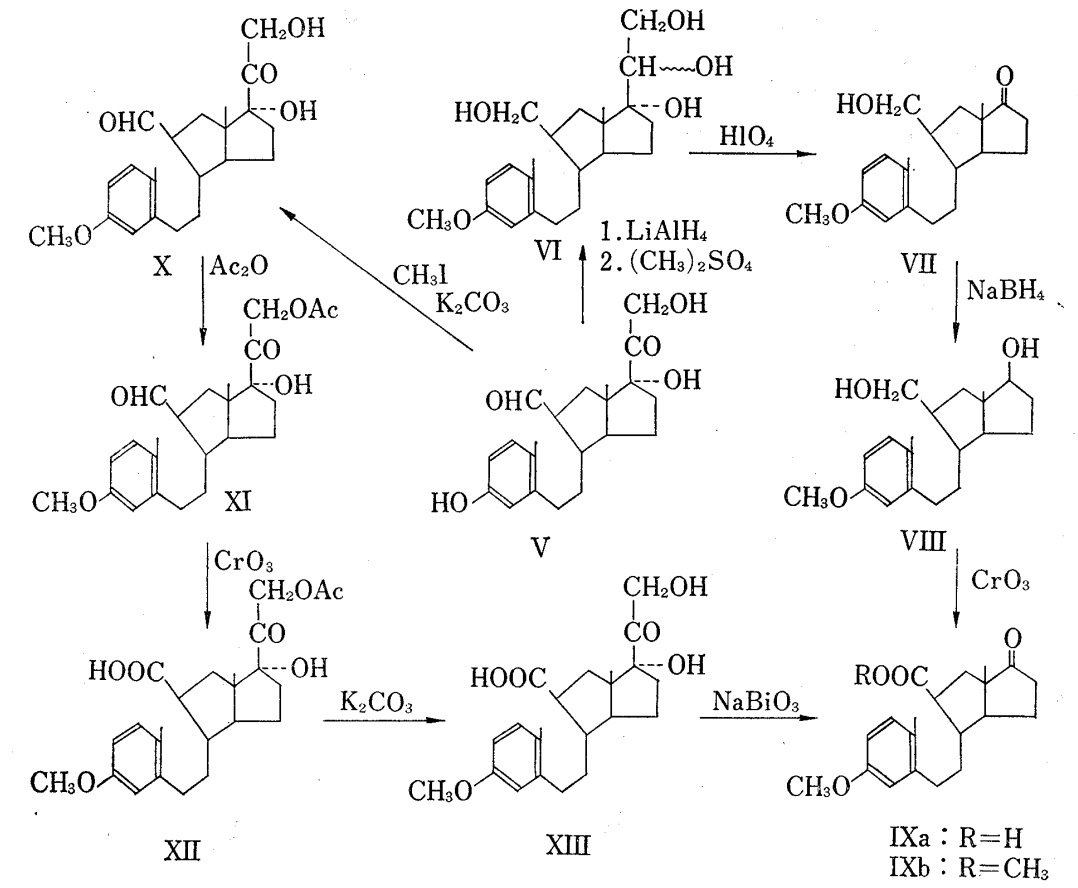


Chart 3

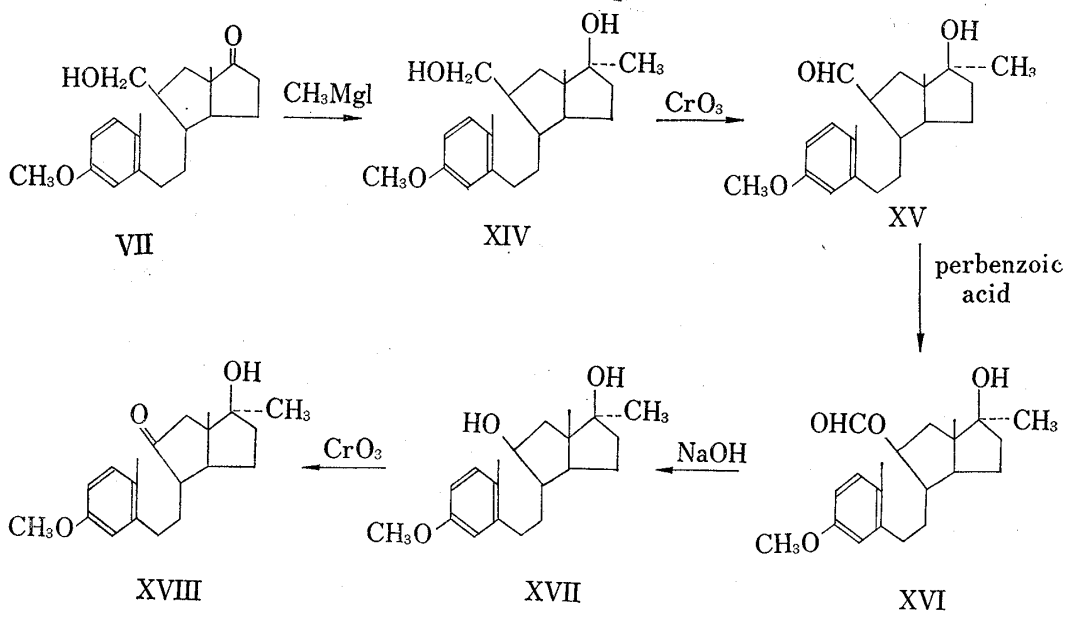


Chart 4

The nature of C-ring was investigated by the experiments shown in Chart 4. The ketone (VII) was methylated with methylmagnesium iodide and oxidized with chromic anhydride to an aldehyde (XV). The aldehyde group of XV was subjected to Baeyer–Villiger reaction to afford formyl ester (XVI), which was hydrolyzed and oxidized successively. The resulting ketone (XVIII) displayed a strong absorption at 1742 cm^{-1} in its IR spectrum to prove that the C-ring of the rearranged product was 5-membered, too.

These data are sufficient to conclude the structure of the diphosphate (A) as shown in Chart 2. Its formation can be rationalized as follows: A new-type dienone-phenol rearrangement accompanied by a pinacolic shift including a cleavage of the bond between positions 9 and 10[5] yielded a diphosphate of C-nor-9,10-secosteroidal aldehyde.

The X-ray analysis of *p*-bromobenzoate of V is completely in agreement with the proposed structure and also provides the configuration around the aldehyde group.

The dienone compound having the 11α-hydroxyl group (XIX) underwent a similar rearrangement to give an aldehyde (XX) as a single product. The aldehyde was found to be the same in all respects as that obtained from 11β-hydroxydienone (XXI). These facts show that the difference in the configuration at C-11 of the starting material makes no difference in the configuration of the product around the aldehyde group. Treatment of prednisolone with acidic catalyst in acetic anhydride and the subsequent hydrolysis gave the normal rearrangement product (XXII)[6] as the main product along with the secosteroidal aldehyde (V) in the yield of less than 10%. When dexamethasone (XXIII) was treated with pyrophosphoryl chloride, no rearrangement was observed due perhaps to the electronically inductive effect of the 9α-fluorine atom.

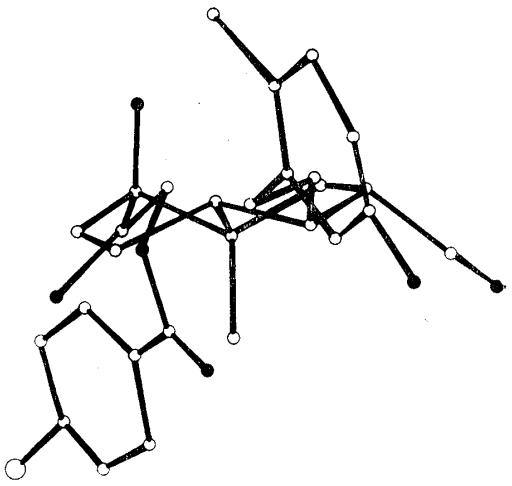


Fig. 4. Crystal Structure of *p*-Bromobenzoate of V Determined by X-Ray Analysis

○: Br ●: O ○: C

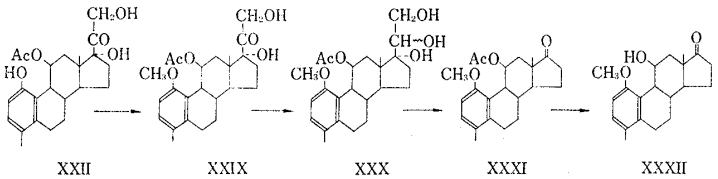
Pharmacology

The compound (V) showed no anti-inflammatory activity in the carrageenin edema and cotton pellet test[7] by oral administration in rats.

The pure toxic diphosphate (A) suppressed the contraction amplitude of the isolated guinea pig auricle even in the concentration of 0.1 μg/ml, and the suppression was removed by washing (Fig. 1). The inhibitory action was not influenced by pretreatment with atropine or neostigmine, indicating that the diphosphate has no cholinergic properties. The activity of the acetylcholinesterase in the homogenate of guinea pig brain was not influenced even in the concentration of 100 μg/ml.

5) The other aromatization reactions leading to 9,10-secosteroid so far reported were either by pyrolysis at 350°[a] or metal-ammonia reduction[b] or heating with zinc in pyridine.[c]
a) B.J. Margerlin and J.A. Hogg, *Tetrahedron*, **2**, 80 (1958); *b*) M. Tanabe, J.W. Chamberlin, and P. Nishimura, *Tetrahedron Letters*, **1961**, 601; *c*) K. Tsuda, S. Nozoe, and K. Okada, *J. Org. Chem.*, **28**, 783, 786 (1963).

6) E.J. Baily, J. Elks, J.F. Oughton, and L. Stephenson, *J. Chem. Soc.*, **1961**, 4535. Baily, *et al.* described the treatment of prednisolone with acidic catalyst in acetic anhydride and proposed the structure (XXII) of the product. We confirmed the structure by converting it to the compound (XXXII) as shown in the following chart and by comparison with the authentic sample obtained by Tsuda's method.[5c]



XXII XXIX XXX XXXI XXXII

7) C.A. Winter, E.A. Risley, and G.W. Nuss, *Proc. Soc. Exp. Biol. Med.*, **111**, 544 (1962); *idem*, *J. Pharmacol. Exp. Ther.*, **141**, 369 (1963).

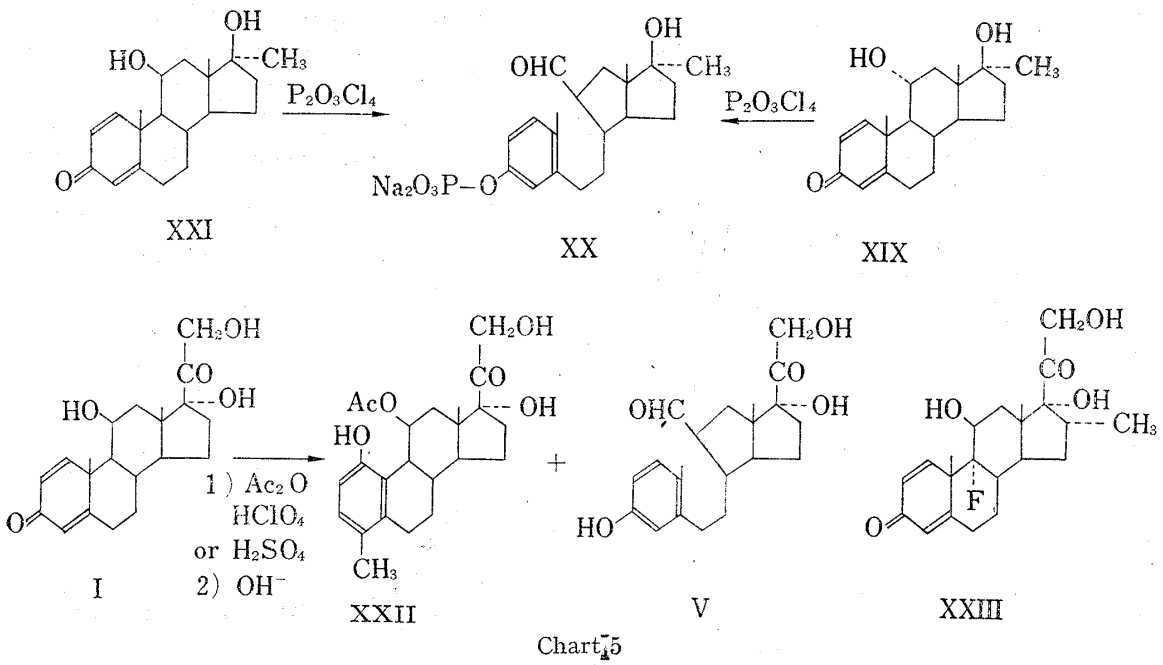


Chart 5

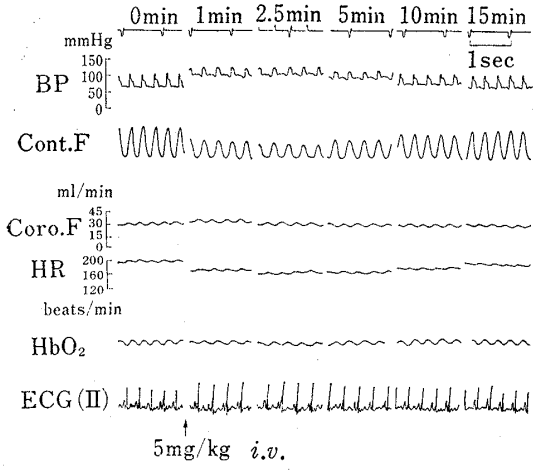


Fig. 5. The Effects of Intravenouly Injected Compound (A) on the Blood Pressure (BP), Right Ventricular Contractile Force (Cont. F), Coronary Sinus Outflow (Coro. F), Heart Rate (HR), Oxyhemoglobin Concentration of Coronary Sinus Blood (HbO_2), and Lead II Electrocardiograms (ECG II) in the Anesthetized Dog

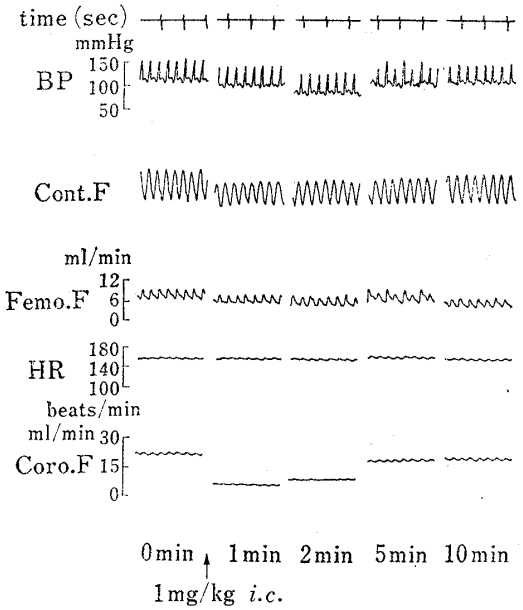


Fig. 6. The Effects of Intra-coronarilly Injected Compound (A) on the Blood Pressure (BP), Left Ventricular Contractile Force (Cont. F), Femoral Blood Flow (Femo. F), Heart Rate (HR) and Coronary Artery Blood Flow (Coro. F) in the Anesthetized Dog

In pentobarbital (30 mg/kg, *i.v.*) anesthetized, open-chest dogs, an intravenous injection of more than 1 mg/kg of the compound produced a rise in the blood pressure and a decrease in the contractile force of the ventricle and heart rate (Fig. 5). The coronary blood flow changed little in spite of the marked rise of the blood pressure. Direct injection into the coronary artery reduced the coronary blood flow and the contractile force (Fig. 6). A similar vasoconstricting action was observed, when it was injected into the femoral artery.

Finally an interesting relation between the structure and the toxic activity was found (Chart 6). Reaction of the aldehyde (V) with pyrophosphoryl chloride gave a new 21-mono-

phosphate (XXIV), which showed a strong toxic activity on the guinea pig auricle, the contractile amplitude of which was suppressed by 25% in the concentration of 10^{-6} g/ml, while the aldehyde (V), the 21-acetate (IV) and the 21-alcohol (XXV) obtained by hydrolysis of IV with potassium carbonate showed no toxic activities. When the 21-alcohol (XXV) was treated with pyrophosphoryl chloride the strongly toxic diphosphate (A) was obtained. These results show that the 21-phosphate group is essential for the toxicity, while the 3-phosphate group has no participation. The disulphate (XXVI) had a slight effect, whereas the succinate (XXVII)[8] exerted a strong effect and the contractile amplitude was suppressed by 40% in the concentration of 10^{-4} g/ml. The carboxylate (XXVIII) which is the oxidation product of the diphosphate (A) with permanganate showed no toxicity.

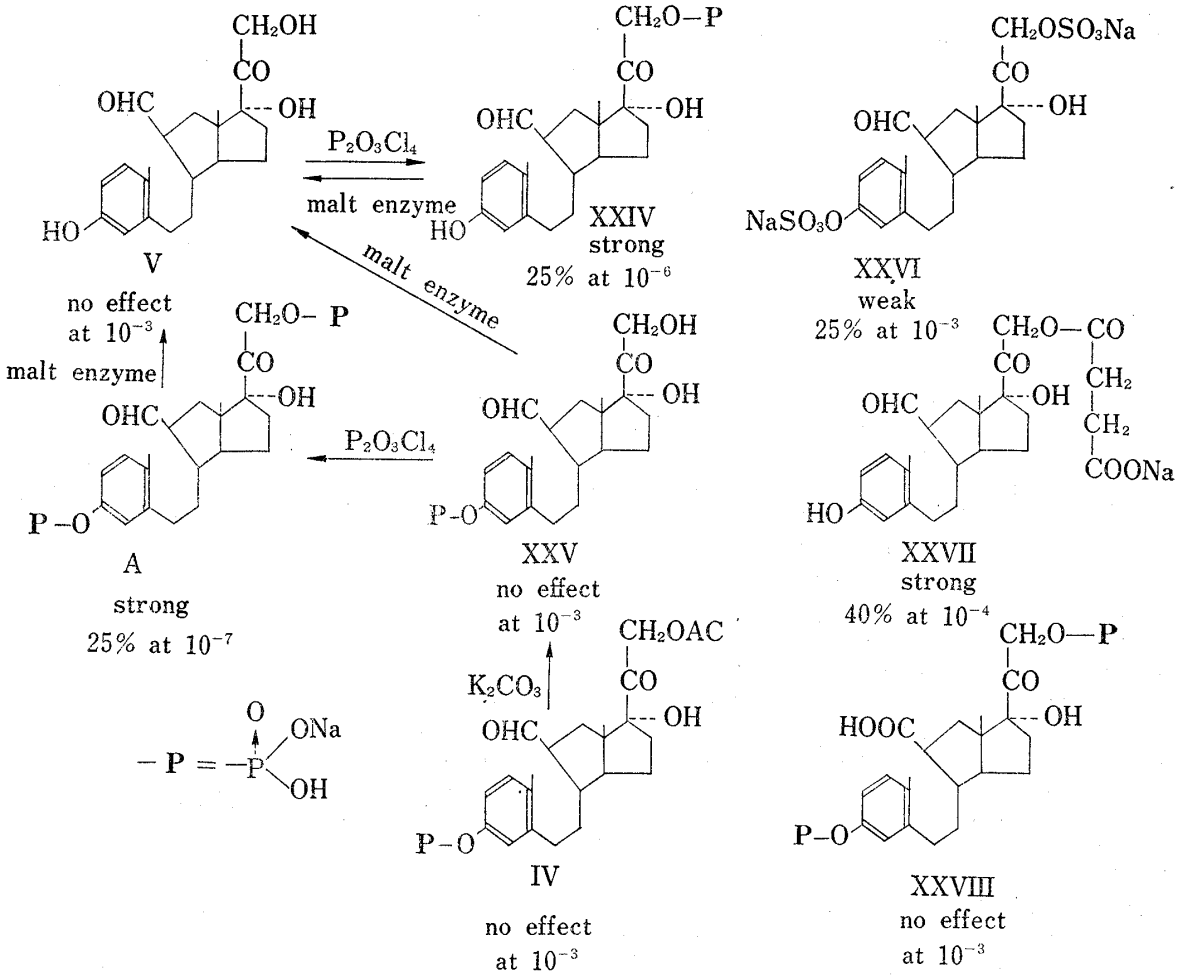


Chart 6

Suppression at a concentration (g/ml) on guinea pig auricle

Experimental[9]

Prednisolone Phosphate (II)——To a solution of prednisolone (250 g) in a mixture of 1.08 liters of tetrahydrofuran (THF) and 2.39 liters of *m*-cresol was gradually added 350 g of pyrophosphoryl chloride with stirring at −40°, and stirring was continued for 3 hr at the same temperature. To this solution was added 2.0 liters of water, keeping the temperature under 0°, and the mixture was stirred with 800 g of charcoal.

8) There is a possibility of contamination with 3,21-disuccinate. See Experimental part.
9) Melting points are uncorrected. NMR spectra were obtained in the specified solvent on a Varian A-60 spectrometer with tetramethylsilane as an internal standard. Jone's reagent was prepared by diluting a solution of 26.72 g of CrO_3 in 23 ml of conc. H_2SO_4 with water to 100 ml; C. Djerassi, R.R. Engle, and A. Bowers, *J. Org. Chem.*, **21**, 1548 (1956).

After removal of most of organic solvent under reduced pressure, the charcoal was filtered and washed with water until the wash water showed pH 4. The charcoal was suspended in 1.0 liter of MeOH and the suspension was adjusted to pH 8.5 with 2N methanolic NaOH solution. After filtration the charcoal was extracted several times with MeOH. The combined MeOH extracts were concentrated and the residue was dried in a desiccator, then dissolved in a small amount of MeOH and filtered. On addition of petroleum ether to this solution 250 g of colorless precipitates were obtained.

Dexamethasone Phosphate——To a solution of dexamethasone (7.8 g) in 100 ml of THF, a solution of pyrophosphoryl chloride (10 g) in 20 ml of THF was added with stirring at −40°. The reaction mixture was kept at the same temperature for 2 hr, diluted with 100 ml of H_2O, and the solvent was evaporated under reduced pressure. After the addition of H_2O (200 ml) dexamethasone dihydrogen phosphate precipitated out of the solution. mp 176—178°. Yield 6.0 g.

Diprednisolone Phosphate——A solution of prednisolone (4.0 g), pyridine (2.0 ml) and THF (50 ml) was added to a solution of $POCl_3$ (3.7 ml) in 30 ml of THF at 10° for 30 min with stirring. The mixture was poured into an aqueous solution of $NaHCO_3$ (20 g). The solvent was evaporated under reduced pressure to precipitate solid substance, which was filtered and recrystallized from MeOH to give 3.0 g of sodium salt of diprednisolone phosphate, mp 235° (decomp.). The sodium salt was shaken with HCl solution and AcOEt, and the organic layer was condensed. The residual solid was recrystallized from AcOEt and MeOH to afford colorless prisms, mp 205°. *Anal.* Calcd. for $C_{12}H_{55}O_{12}P \cdot 2H_2O$: C, 61.60; H, 7.26; P, 3.78. Found: C, 61.05; H, 7.24; P, 3.75. The melting point did not depress on admixture with the sample of diprednisolone-(21) phosphate which was prepared by the reaction of disodium prednisolone-(21) phosphate and prednisolone 21-iodide in dimethy formamide (DMF) at 100° for 30 min.

From the aqueous filtrate of sodium diprednisolone phosphate, there was obtained 1.0 g of sodium prednisolone phosphate by the procedure described above. Changes of reaction condition caused little difference in the ratio of mono- and diphosphate in the products.

Synthesis of A and IV——To a suspension of 36 g of prednisolone (I) in 500 ml of THF cooled at −30° was added 50 g of pyrophosphoryl chloride with stirring, then the temperature of the reaction mixture was raised to 10° and stirring was continued for 3 hr. To this mixture was added 100 ml of water and most of THF was removed under reduced pressure. After dilution with 400 ml of water, 100 g of active charcoal was added to adsorb the product. The charcoal was filtered, washed with water until the wash water showed pH 4, then suspended in 300 ml of MeOH and the suspension was adjusted to pH 8 with aq. NaOH. The charcoal was extracted 3 times with 200 ml of MeOH. The combined extracts were concentrated to give 30 g of a pale yellow syrup which contained compound A. In the same way as described above, 30 g of IV was prepared from 32 g of prednisolone acetate (III).

Hydrolysis of A with Malt Enzyme——To a solution of the toxic compound (A) (0.5 g) in 100 ml of water was added malt enzyme (Diastase, 0.5 g) and the mixture was shaken for a few min, then kept overnight at room temperature. The reaction mixture was shaken with AcOEt, and the AcOEt layer was dried over Na_2SO_4, evaporated to give 0.3 g of a yellow solid, which was crystallized by treatment with aqueous MeOH. Recrystallization from aqueous MeOH gave colorless needles melting at 92.4°. *Anal.* Calcd. for $C_{21}H_{28}O_5 \cdot H_2O$: C, 66.67; H, 7.93. Found: C, 66.52; H, 7.91. IR ν_{max}^{Nujol} cm^{-1}: 3450, 3250, 2700, 1699, 1611, 1581, 1500, 820. NMR (τ, in $CDCl_3$): 9.42 (3H, singlet, 18-CH_3), 7.79 (3H, singlet, C_6H_5–CH_3), 5.52, 5.77 (2H, doublet, J=17.5 Hz, 21-CH_2), 3.42 (2H, aromatic H), 3.0 (1H, doublet, J=7.5 Hz, aromatic H), 0.22 (1H, CHO), semicarbazone: mp 222°. *Anal.* Calcd. for $C_{22}H_{31}O_5N_3$: C, 63.31; H, 7.43; N, 10.07. Found: C, 63.12; H, 7.32; N, 9.79.

Hydrolysis of IV with Malt Enzyme——Hydrolysis of IV (5 g) was carried out in a similar manner as above and the products were chromatographed on silica gel (100 g). The fraction eluted with benzene–AcOEt (4: 1) gave 2.2 g of a monoacetate, which was recrystallized from ether–hexane. mp 148—150°. *Anal.* Calcd. for $C_{23}H_{30}O_6$: C, 68.66; H, 7.46. Found: C, 68.65; H, 7.41. IR ν_{max}^{Nujol} cm^{-1}: 3350, 2700, 1720, 1605, 1583, 1498. NMR (τ, in $CDCl_3$): 9.40 (3H, singlet, 18-CH_3), 7.82 (6H, singlet, $OCOCH_3$, C_6H_5–CH_3), 5.34, 4.78 (2H, doublet, J=17.5 Hz, 21-CH_2), 3.44 (2H, aromatic H), 3.05 (1H, doublet, J=8 Hz, aromatic H), 0.2 (1H, CHO). The fraction eluted with benzene–AcOEt (2: 1) afforded 2.2 g of crystals. mp 93° (V).

Alkaline Hydrolysis of the Monoacetate——To a solution of the acetate (0.1 g) in 2 ml of MeOH was added 1.5 ml of 10% KOH solution and the solution was shaken vigorously. After 15 min, 50 ml of water was added. The solution was adjusted to pH 3.4 with AcOH and allowed to stand to separate colorless needles out. Recrystallization from aqueous MeOH gave 0.05 g of V.

17α,20,21-Trihydroxy-9-hydroxymethyl-3-methoxy-9,10-seco-C-norpregna-1,3,5(10)-triene (VI)——To a solution of $LiAlH_4$ (0.3 g) in 20 ml of THF was gradually added a solution of V (0.3 g) in 20 ml of THF with stirring and the mixture was refluxed for 4 hr. After cooling, AcOEt (5 ml) and then dil. H_2SO_4 were added, and the mixture was shaken with AcOEt. The organic layer was washed with water, dried over Na_2SO_4, and evaporated to give 0.1 g of an oily material, which was dissolved in 20 ml of MeOH, and methylated with Me_2SO_4 (0.12 g) and 50% KOH at 50°. After heating for 3 hr, the reaction mixture was diluted with water and extracted with AcOEt. The AcOEt extract was washed with water, dried over Na_2SO_4, evaporated to give 0.1 g of crude VI as an oil. NMR (τ, in $CDCl_3$): 6.30 (3H, singlet, OCH_3), 7.78 (3H, singlet, C_6H_5–CH_3),

8.80 (3H, singlet, 18-CH_3).

9β-Hydroxymethyl-3-methoxy-9,10-seco-C-norandrosta-1,3,5(10)-trien-17-one (VII)——To a solution of VI (1 g) in 15 ml of EtOH–H_2O (1: 1) was added $NaIO_4$ (1 g) and the mixture was stirred for 10 min at room temperature. This solution was diluted with 100 ml of water, and extracted with AcOEt. The organic layer was washed with water, dried over Na_2SO_4, evaporated to leave 0.9 g of an oily residue, which was chromatographed on silica gel. Elution with benzene–AcOEt (2: 1) afforded 0.5 g of VII. Recrystallization from EtOH–H_2O gave colorless needles. mp 84—86°. *Anal.* Calcd. for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.95; H, 8.68. IR ν_{max}^{Nujol} cm^{-1}: 3450, 1735, 1250. $\nu_{max}^{CS_2}$ cm^{-1}: 1743. NMR (τ, in $CDCl_3$): 8.91 (3H, singlet, 18-CH_3), 7.75 (3H, singlet, C_6H_5–CH_3), 6.20 (3H, singlet, OCH_3), 2.8, 3.4 (3H, aromatic H), $[\alpha]_D^{20}$ +78.8 (c= 1, in $CHCl_3$).

9β-Hydroxymethyl-3-methoxy-9,10-seco-C-norandrosta-1,3,5(10)-trien-17-ol (VIII)——To a solution of VII (0.25 g) in MeOH was added 0.05 g of $NaBH_4$ and stirring was continued for 1 hr at −5°. After adding 2 drops of AcOH and 50 ml of water, the mixture was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4, and evaporated to give an oily residue which was purified by chromatography on silica gel followed by recrystallization from aqueous MeOH. mp 95—97°. *Anal.* Calcd. for $C_{20}H_{30}O_3 \cdot 1/3H_2O$: C, 74.03; H, 9.52. Found: C, 74.02; H, 9.39. IR ν_{max}^{Nujol} cm^{-1}: 3250, no C=O absorption.

3-Methoxy-17-oxo-9,10-seco-C-norandrosta-1,3,5(10)-trien-9β-carboxylic Acid (IXa), Methyl Ester (IXb)——To a solution of VIII (0.13 g) in acetone was gradually added Jones' reagent (0.6 ml). After a few minutes, the excess of Jones' reagent was decomposed with MeOH. This mixture was diluted with water, extracted with AcOEt, and the extract was washed with water, dried over Na_2SO_4 and evaporated to give 0.1 g of IXa. IR $\nu_{max}^{liq.}$ cm^{-1}: 1700, 1735. Treatment of ether solution of IXa (6.128 g) with CH_2N_2 followed by chromatography on silica gel yielded 0.07 g of the methyl ester (IXb) of IXa as an yellow oil. IR $\nu_{max}^{liq.}$ cm^{-1}: 1730, 1740, 1609, 1580, 1500. NMR (τ, in $CDCl_3$): 6.32 (3H, singlet, OCH_3), 6.26 (3H, singlet, $COOCH_3$).

9β-Formyl-17α,21-dihydroxy-3-methoxy-9,10-seco-C-norpregna-1,3,5(10)-trien-20-one (X)——To a solution of V (1 g) in 25 ml of acetone was added 2 g of K_2CO_3 and 10 g of MeI and the mixture was refluxed for 40 hr. After evaporation of the solvent, the residue was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4, and evaporated to leave a crystalline material (0.6 g), which was purified by chromatography on silica gel and recrystallized from ether–hexane. mp 105°. *Anal.* Calcd. for $C_{22}H_{30}O_5$: C, 70.59; H, 8.02. Found: C, 70.63; H, 8.45. IR ν_{max}^{Nujol} cm^{-1}: 3400, 2700, 1710. NMR (τ, in $CDCl_3$): 4.25 (3H, singlet, OCH_3), 0.22 (1H, CHO).

21-Acetoxy-9β-formyl-17-hydroxy-3-methoxy-9,10-seco-C-norpregna-1,3,5(10)-trien-20-one (XI)——A solution of X (0.2 g) and Ac_2O (0.2 ml) in 3 ml of pyridine was allowed to stand at room temperature for 1 hr. The solution was successively washed with dil. HCl, $NaHCO_3$ solution, water and dried over Na_2SO_4. On evaporating ether, 0.15 g of a crystalline material was obtained and purified by recrystallization from ether–hexane to give colorless prisms. mp 110°. *Anal.* Calcd. for $C_{24}H_{32}O_6$: C, 69.28; H, 7.91. Found: C, 69.23; H, 7.69. IR ν_{max}^{Nujol} cm^{-1}: 3430, 2700, 1740, 1720. NMR (τ, in $CDCl_3$): 7.83 (3H, singlet, $OCOCH_3$), 7.73 (3H, singlet, C_6H_5–CH_3), 0.22 (1H, CHO). Semicarbazone: mp 182. *Anal.* Calcd. for $C_{25}H_{35}O_6N_3$: C, 63.42; H, 7.40; N, 8.88. Found: C, 63.07; H, 7.40; N, 8.26.

21-Acetoxy-17α-hydroxy-3-methoxy-20-oxo-9,10-seco-C-norpregna-1,3,5(10)-triene-9β-carboxylic Acid (XII)——To a solution of XI (0.4 g) in 10 ml of acetone was added 0.4 ml of Jones' reagent and the mixture was stirred for 10 min. After addition of water the mixture was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4, and evaporated to leave 0.3 g of a crystalline residue, which was recrystallized from ether–hexane. mp 86—87°. *Anal.* Calcd. for $C_{24}H_{32}O_7$: C, 66.67; H, 7.47. Found: C, 66.49; H, 7.45. IR ν_{max}^{Nujol} cm^{-1}: 3500, 2500, 1730 (broad).

17α,21-Dihydroxy-3-methoxy-20-oxo-9,10-seco-C-norpregna-1,3,5(10)-trien-9β-carboxylic Acid (XIII)——To a solution of XII (0.2 g) in 2 ml of MeOH was added 10% K_2CO_3 solution and the mixture was stirred for 30 min at room temperature, then diluted with water and, after adjusting the pH to 2, extracted with water. The extract was dried over Na_2SO_4, evaporated to give a crystalline residue which was purified by recrystallization from ether–hexane. Yield 0.1 g. mp 164—165°. *Anal.* Calcd. for $C_{22}H_{30}O_6$: C, 67.69; H, 7.69. Found: C, 67.38; H, 7.48.

3-Methoxy-17-oxo-9,10-seco-C-norandrosta-1,3,5(10)-triene-9β-carboxylic Acid (IXa)——To a solution of XIII (0.2 g) in 6 ml of AcOH and 4 ml of water was added $NaBiO_3$ (1.2 g) and the solution was stirred for 2 hr at 45°. The reaction mixture was filtered and the filtrate was diluted with water, then extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and evaporated to give 0.2 g of an oily material, the IR spectrum of which was identical with that of IXa obtained from VIII.

9β-Hydroxymethyl-3-methoxy-17α-methyl-9,10-seco-C-norandrosta-1,3,5(10)-trien-17β-ol (XIV)——The Grignard reagent prepared from 0.5 g of Mg and 1.0 g of MeI was added to a solution of VII (0.7 g) in ether and the solution was refluxed with stirring for 1 hr. After cooling NH_4Cl was added to the solution, and the mixture was extracted with ether. The ether extract was washed with water, dried over Na_2SO_4, and evaporated to give an oily residue which was chromatographed on silica gel. The fraction eluted with benzene–AcOEt (3: 1) afforded 0.5 g of an yellow oily residue. IR $\nu_{max}^{liq.}$ cm^{-1}: 3450, 3300, 1616, 1590, 1505. NMR: (τ, in $CDCl_3$), 9.98 (3H, singlet, 18-CH_3), 8.63 (3H, singlet, C_{17}–CH_3), 7.75 (3H, singlet, C_6H_5–CH_3),

6.20 (3H, singlet, OCH_3).

9β-Formyl-3-methoxy-17α-methyl-9,10-seco-C-norandrosta-1,3,5(10)-trien-17β-ol (XV)——To a solution of XIV (0.5 g) in 30 ml of acetone was added 0.5 ml of Jones' reagent. After decomposing the excess Jones' reagent with MeOH, the reaction mixture was diluted with water, and extracted with AcOEt. The AcOEt extract was washed with water, dried over Na_2SO_4, and evaporated to give an oily residue, which was purified by chromatography on silica gel. Yield 0.25 g. IR $\nu_{max}^{liq.}$ cm^{-1}: 2720, 1720. NMR (τ, in $CDCl_3$): 0.15 (1H, CHO). Semicarbazone, mp 112°. *Anal.* Calcd. for $C_{22}H_{33}O_3N_3$: C, 68.21; H, 8.52; N, 10.85. Found: C, 68.36; H, 8.61; N, 10.71.

3-Methoxy-17α-methyl-9,10-seco-C-norandrosta-1,3,5(10)-triene-9,17β-diol (XVII)——To a solution of XV (1 g) in benzene was added 71 ml of perbenzoic acid solution (0.02387 g/ml of benzene) and the solution was allowed to stand at room temperature overnight. The solution was washed with 0.1N $Na_2S_2O_4$ and then with water, dried over Na_2SO_4, and evaporated to give an oily residue, from which pure XVI was separated by chromatography on silica gel. The XVI thus obtained was dissolved in MeOH–THF, and to this solution was added 0.5 ml of 30% NaOH. The solution was stirred at room temperature for 5 min, then poured into water, and extracted with benzene. The combined extracts were washed with water, dried over Na_2SO_4, and evaporated to give 0.08 g of XVII.

17β-Hydroxy-3-methoxy-17α-methyl-9,10-seco-C-norandrosta-1,3,5(10)-trien-9-one (XVIII)——To a solution of XVII (0.08 g) in 10 ml of acetone was added 0.1 ml of Jones' reagent. The excess of oxidizing reagent was decomposed with MeOH, then the mixture was diluted with water and extracted with ether. The combined extracts were washed with water, dried over Na_2SO_4, and evaporated to give an oily residue which was purified by chromatography followed by recrystallization from ether to give 0.05 g of XVIII. mp 128—130°. *Anal.* Calcd. for $C_{20}H_{28}O_3$: C, 75.91; H, 8.91. Found: C, 75.31; H, 8.85. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1742, 1610, 1576. NMR (τ, in $CDCl_3$): 9.09 (3H, singlet, 18-CH_3), 8.72 (3H, singlet, C_{17}–CH_3), 7.84 (3H, singlet, C_6H_5–CH_3), 6.35 (3H, singlet, OCH_3), 3.1, 3.7 (3H, aromatic H).

Prednisolone *p*-Bromobenzoate——To a solution of prednisolone (1 g) in 20 ml of THF and 1 ml of pyridine was added a solution of *p*-bromobenzoyl chloride (3 g) in THF under ice-cooling and the mixture was allowed to stand for 3 hr at room temperature. The mixture was poured into water to give colorless precipitates (4 g) which were collected by filtration. mp 215—220°.

***p*-Bromobenzoate of V**——To a solution of the *p*-bromobenzoate of prednisolone (0.4 g) in 10 ml of THF was added 1.5 ml of pyrophosphoryl chloride under ice cooling. Then the reaction mixture was stirred for 3 hr at room temperature. The reaction mixture was cooled again in ice bath and to this mixture was slowly added 50 ml of water, then 2 g of charcoal with stirring. The charcoal was collected by filtration, washed with water until the wash water showed pH 3. The charcoal was suspended in MeOH and the suspension was adjusted to pH 8.5 with 2N methanolic NaOH solution. After filtration, the charcoal was extracted several times with MeOH and the combined extracts were evaporated to dryness. The residue (0.6 g) was dissolved in 50 ml of water and to this solution was added 2 g of malt enzyme. After stirring followed by standing for 5 hr, the mixture was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4, and evaporated to give 0.25 g of an oily product. This oil was chromatograpged on a column of silica gel (15 g) using benzene–ether (5: 1) as eluant to give 0.1 g of crystals which were recrystallized from AcOEt–hexane. mp 180—181°. *Anal.* Calcd. for $C_{28}H_{31}O_6Br$: C, 61.88; H, 5.71; Br, 14.73. Found: C, 62.23; H, 5.75; Br. 16.72.

Dienone-phenol Rearrangement of 11α,17β-Dihydroxy-17α-methylandrosta-1,4-dien-3-one (XIX)——To a solution of XIX (0.6 g) in 12 ml of THF was added 1.8 ml of pyrophosphoryl chloride with stirring under ice cooling and the mixture was kept at the same temperature for 1 hr. After additional 2 hr of stirring at room temperature, the mixture was diluted with 50 ml of water, then 2.4 g of charcoal was added. After working up in a similar manner with malt enzyme as described above 0.9 g of an oily material (XX) was obtained which was purified by chromatography on silica gel using benzene–AcOEt (9: 1) as eluant. IR $\nu_{max}^{liq.}$ cm^{-1}: 3450, 2750, 1720, 1620, 1598, 1510. NMR (τ, in CCl_4): 9.0 (6H, singlet, 13-CH_3, 17-CH_3), 7.91 (3H, singlet, C_6H_5–CH_3), 3.18, 3.7 (3H, aromatic H), 0.45 (1H, CHO).

The 11β-hydroxy isomer (XXI) was treated with pyrophosphoryl chloride under the same condition to give an oily product which was identical with that of the rearranged product (XX) in the IR, NMR spectra and the *Rf* value of TLC.

Dienon-phenol Rearrangement of Prednisolone with Ac_2O–$HClO_4$——To a solution of prednisolone (I, 3 g) in 80 ml of $CHCl_3$ was added 20 ml of Ac_2O and 0.05 ml of $HClO_4$ (60%), and the solution was stirred for 1 hr at 0°, then for 1 hr at room temperature. The reaction mixture was poured into ice-water, and the $CHCl_3$ layer was separated, washed with 5% Na_2SO_4 solution, then with water, dried over Na_2SO_4, and evaporated to give an oily residue. The starting material (I) was removed by chromatography on silica gel, then the products (2 g) were dissolved in 150 ml of MeOH. To this solution was added 140 ml of 0.1N NaOH solution and the solution was stirred for 5 min at room temperature. The mixture was diluted with water and extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4, and evaporated to give an oily residue, which was crystallized on treatment with MeOH. Recrystallization from MeOH gave 0.4 g of colorless needles (XXII). mp 215—219°. *Anal.* Calcd. for $C_{23}H_{30}O_6 \cdot H_2O$: C, 65.69; H, 7.67. Found:

C, 65.86; H, 7.65.

The filtrate of recrystallization was concentrated to give an oily residue which was chromatographed on silica gel. The fraction eluted with benzene–AcOEt (2: 1) gave 0.1 g of V. The compound (V) was also separated from the residue by extraction with hot water. Prednisolone acetate (III) yielded the same products on treatment with Ac_2O and 60% $HClO_4$.

21-Phosphate of V——To a solution of V (0.35 g) in 10 ml of THF was added 1.2 ml of pyrophosphoryl chloride dropwise with stirring at −35°. The mixture was kept at the same temperature for 7 hr, diluted with 50 ml of water at 0°, then 1.5 g of charcoal was added. Then the charcoal was filtered and washed with water until the wash water showed pH 4. The charcoal was suspended in 50 ml of MeOH and the suspension was adjusted to pH 8.5 with 2N methanolic NaOH solution. After filtration the charcoal was extracted several times with MeOH. The combined MeOH extracts were concentrated and the residue was dried in a vacuum desiccator, then dissolved in a small amount of MeOH and filtered. On addition of petroleum ether to the MeOH solution, 0.25 g of colorless precipitates were obtained. This product was hydrolyzed back to V by malt enzyme in a similar manner as described above.

Hydrolysis of IV——To a solution of IV (2 g) in 20 ml of MeOH was added 10% K_2CO_3 and the solution was shaken well. After standing at room temperature overnight the solution was acidified by dil. HCl, then 1 g of charcoal was added and the mixture was worked up as stated above to give 1.5 g of XXV.

Phosphorylation of XXV——XXV (0.3 g) was dissolved in 25 ml of THF and phosphorylated by the procedure stated in the case of phosphorylation of V to give 0.2 g of A. Identification was made by comparison of the behavior on TLC and hydrolysis with malt enzyme to give V.

Disulfate (XXVI) of V——To a solution of V (1 g) in pyridine (15 ml) and $CHCl_3$ (5 ml) was added in portions 1.5 g of $ClSO_3H$ with stirring under ice cooling. After 2 hr, the solution was acidified to pH 2 with 50 ml of 20% H_2SO_4, then 5 g of active charcoal was added to adsorb the product and the mixture was worked up according to the procedure described in the preparation of A to give 0.7 g of colorless powder.

Succinate (XXVII) of V——To a solution of V (1 g) in 10 ml of pyridine was added 1.5 g of succinic anhydride and the mixture was stirred at 50° for 3 hr, then poured into water. The precipitates formed were extracted with AcOEt and the AcOEt solution was shaken with aqueous $NaHCO_3$ solution. The water layer was separated, acidified with 10% H_2SO_4 and extracted with AcOEt, washed with water, dried over Na_2SO_4, and evaporated to give 1 g of powder which showed 2 spots on TLC, indicating that the product was a mixture of mono- and di-succinate. This mixture was dissolved in MeOH and neutralized with MeONa–MeOH solution. After evaporation of the solution to dryness, the residual powder was dissolved in a small amount of MeOH and reprecipitated with acetone.

Permanganate Oxidation of Compound A——To a solution of the compound A (0.5 g) in 8 ml of water was added dropwise a solution of $KMnO_4$ (0.13 g) in 6 ml of water with stirring at room temperature. After 1 hr, the precipitated MnO_2 was filtered off, washed with water, and the combined filtrates were acidified with dil. HCl. To this solution was added active charcoal to adsorb the product and the mixture was worked up according to the procedure described in the preparation of the compound A. The solution of this oxidation product (0.1 g) in 5 ml of water was treated with malt enzyme (0.1 g, room temperature, overnight), extracted with AcOEt, dried over Na_2SO_4, and evaporated to give 0.1 g of an oily material. This was chromatographed on a column of silica gel. The fraction eluted with benzene–ether (2: 1) gave 0.05 g of an oily material. IR $\nu_{max}^{liq.}$ cm^{-1}: 2600—3500, 1725, 1708. NMR (τ, in pyridine): 9.6 (3H, singlet, 13-CH_3), 7.84 (3H, singlet, C_6H_5-CH_3), 5.46, 4.95 (1H, 1H, doublet, J=19 Hz, 21-CH_2).

11β-Acetoxy-17α,21-dihydroxy-1-methoxy-4-methylpregna-1,3,5(10)-trien-20-one (XXIX)——To a solution of XXII (1 g) in 30 ml of acetone was added K_2CO_3 (2 g) and CH_3I (10 g), and the mixture was refluxed for 48 hr. The reaction mixture was diluted with water and extracted with AcOEt. The AcOEt extract was washed with water, dried over Na_2SO_4, and evaporated to give a crystalline residue, which was purified by column chromatography on silica gel and recrystallization from ether–*n*-hexane to give 0.8 g of XXVI. mp 225—226°. *Anal.* Calcd. for $C_{24}H_{32}O_6$: C, 69.21; H, 7.74. Found: C, 69.19; H, 7.82. IR ν_{max}^{Nujol} cm^{-1}: 3350, 1733, 1710. NMR (τ, in $CDCl_3$), 9.25 (3H, singlet, 18-CH_3), 7.92 (3H, singlet, C_6H_5-CH_3), 6.39 (3H, singlet, OCH_3).

11β-Acetoxy-1-methoxy-4-methylpregna-1,3,5(10)-triene-17α,20,21-triol (XXX)——To a solution of XXIX (0.6 g) in 20 ml of MeOH was added 0.15 g of $NaBH_4$ and the solution was stirred for 30 min at room temperature. Then the solution was diluted with water, extracted with AcOEt and the AcOEt solution was washed with water, dried over Na_2SO_4 and evaporated to give 0.5 g of colorless product.

11β-Acetoxy-1-methoxy-4-methylestra-1,3,5(10)-trien-17-one (XXXI)——To a solution of XXX (0.5 g) in 20 ml of EtOH was added a solution of $NaIO_4$ (0.8 g) in 5 ml of water and the solution was stirred for 30 min at room temperature. The reaction mixture was diluted with water, extracted with ether, and the ether solution was washed with water, dried over Na_2SO_4, evaporated to give a crystalline residue. Recrystallization from hexane afforded 0.3 g of colorless prisms. mp 133—134°. *Anal.* Calcd. for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 73.84; H, 7.98. IR ν_{max}^{Nujol} cm^{-1}: 1740. NMR (τ, in $CDCl_3$): 9.01 (3H, singlet, 18-CH_3), 8.32 (3H, singlet, $OCOCH_3$), 7.89 (3H, singlet, C_6H_5-CH_3), 6.36 (3H, singlet, OCH_3), 3.55 (2H, aromatic H, 11-CH), 3.16 (1H, doublet, J=8 Hz, aromatic H).

11β-Hydroxy-1-methoxy-4-methylestra-1,3,5(10)-trien-17-one (XXXII)——XXXI (0.4 g) was dissolved in 5% methanolic KOH solution and refluxed for 2 hr. The precipitates obtained by adding water to the reaction mixture were collected by filtration. Recrystallization from aq. MeOH afforded colorless needles. mp 210—212°. Identification was made by mixing melting point analysis and comparison of the IR, NMR spectra with those of the authentic sample prepared by the method reported by Tsuda, *et al.*[5]

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