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STUDIES ON A-NORSTEROIDS—VI[1]

DIRECTING EFFECTS OF THE C_{11}-SUBSTITUENTS ON THE ADDITION OF OSMIUM TETROXIDE TO STEROIDAL $\Delta^{1,4}$-3-KETONES

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Abstract—The effects of C_{11}-substituents on the hydroxylation of $\Delta^{1,4}$-3-oxosteroids with osmium tetroxide, have been examined. The 11α-substituents, such as 11α-hydroxy, 11α-acetoxy or 11α-methyl-11β-hydroxy groups, result in the selective addition of osmium tetroxide at the Δ^4 double bond, giving 4β,5β-dihydroxy-Δ^1-3-oxosteroids. On the other hand, the 11-oxo yields exclusively 1α,2α-dihydroxy-Δ^4-3-oxosteroids.

Oxidation of the 1α,2α-dihydroxy-Δ^4-3-oxosteroids having the 11-oxo group with manganese dioxide yields the corresponding A-nor-$\Delta^{3(5)}$-1,2-dioxosteroids, which, however, tend to form the hydrate at the C_1-oxo group.

PREVIOUSLY, in relation to the synthesis of A-norsteroid derivatives,[2] several steroidal $\Delta^{1,4}$-3-ketones (A) have been hydroxylated with osmium tetroxide in pyridine. Of the seven starting materials (A), six have no substituent at the C_{11}-position and one is prednisolone acetate, which has the 11β-hydroxy group. All the compounds yield in approximately equal amounts the two isomeric products, 1α,2α-dihydroxy-Δ^4-3-ones (B) and 4β,5β-dihydroxy-Δ^1-3-ones (C),[2,3] respectively. The (B) isomers on

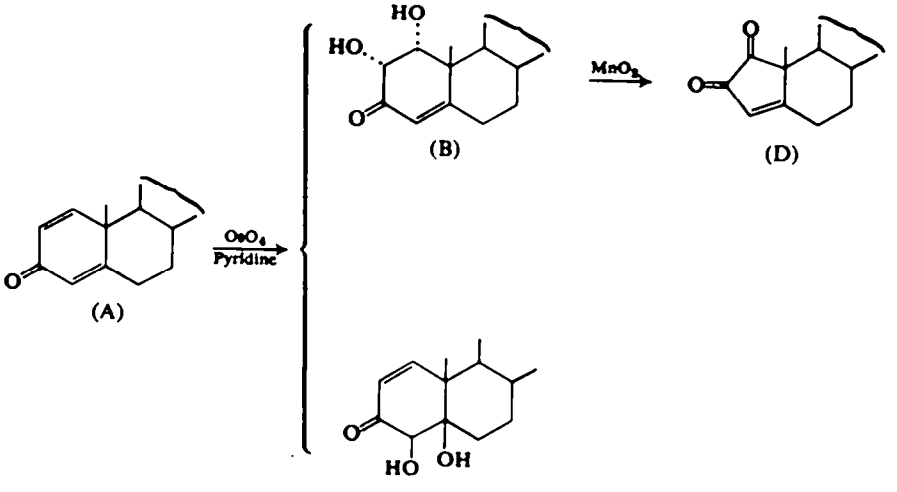


Chart 1

[1] Part V: K. Yoshida and T. Kubota, *Chem. Pharm. Bull.* in press.
[2] T. Kubota, K. Yoshida, F. Hayashi and K. Takeda, *Chem. Pharm. Bull.* 13, 50 (1965).
[3] T. Kubota, K. Yoshida and F. Watanabe, *Chem. Pharm. Bull.* in press.

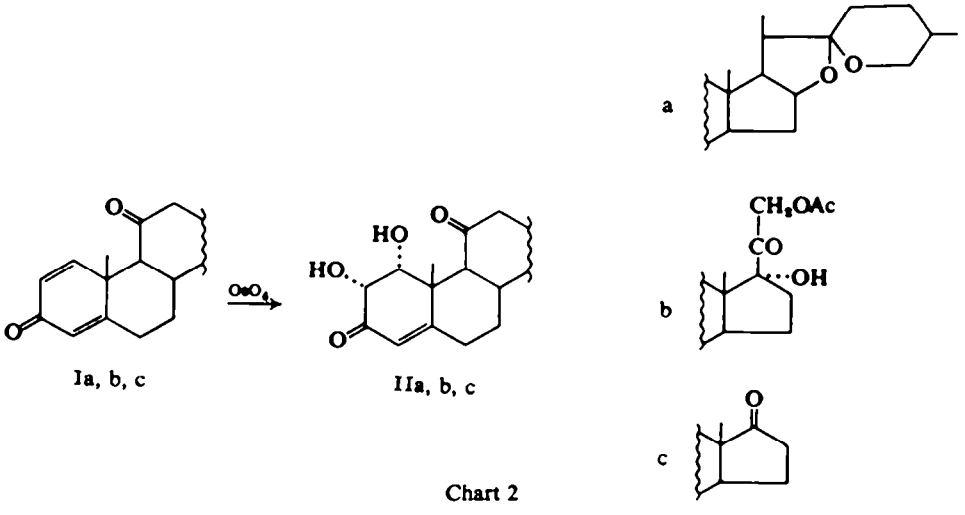
treatment with manganese dioxide have been smoothly converted into the corresponding A-nor-$\Delta^{3(5)}$-1,2-dioxosteroids (D).

On the other hand, it has been reported[4] that the osmium tetroxide hydroxylation of prednisone-BMD—a $\Delta^{1,4}$-3-oxosteroid having the 11-oxo group—yields exclusively the 1,2-dihydroxy-Δ^4-3-one. Examination of molecular models reveals that, while 11β-substituents should have little effect on the $\Delta^{1,4}$-3-oxo system, the 11-oxo group or 11α-substituents being located close to the C_1-proton could have some effect. In fact, abnormalities of the $\Delta^{1,4}$-3-oxo system in the presence of the 11-oxo group and 11α-substituents have been recognized in the studies on UV absorption,[5] CD[6] and NMR.[7]

On this basis the results reported[4] are acceptable but as no explanation was offered, the directing effects of C_{11}-substituents on the addition of osmium tetroxide to steroidal $\Delta^{1,4}$-3-ketones has been examined and also C_{11}-substituted A-norsteroid derivatives have been prepared by oxidation of the 1,2-dihydroxylated products with manganese dioxide as described earlier.[2]

25D-Spirosta-1,4-diene-3,11-dione (Ia), prednisone acetate (Ib) and androsta-1,4-diene-3,11,17-trione (Ic) were selected as starting materials. These, except commercially available prednisone acetate (Ib), were prepared by 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) oxidation of the corresponding 5α-3-oxo- or Δ^4-3-oxosteroid. The $\Delta^{1,4}$-3-oxosteroids having the 11-oxo group (Ia-Ic) were treated with osmium tetroxide as described previously,[2] and the products were chromatographed on silica gel. All three compounds afforded only the 1α,2α-dihydroxy-Δ^4-3-one confirmed by its NMR spectrum showing signals of one olefinic proton and two protons on the OH-bearing carbons. No 4,5-dihydroxylated product was detected. The OH groups introduced were assigned the α-configuration on the basis of previous results.[2]

11α-Hydroxy- (Id), 11α-acetoxy- (Ie) and 11α-methyl-11β-hydroxy- (If) derivatives



Ia, b, c IIa, b, c

Chart 2

[4] R. Hirschmann, G. A. Bailey, R. Walker and J. M. Chemerda, *J. Amer. Chem. Soc.* **81**, 2822 (1959).
[5] L. L. Engel, *Physical Properties of the Steroid Hormones* p. 97. Pergamon Press (1963).
[6] G. Snatzke, *Tetrahedron* **21**, 439 (1965).
[7] K. Tori, unpublished work in this laboratory.

of pregna-1,4-diene-3,20-dione were selected as the $\Delta^{1,4}$-3-oxosteroids with 11α-substituents. Oxidation of 11α-acetoxyprogesterone with DDQ afforded 11α-acetoxypregna-1,4-diene-3,20-dione (Ie), which on saponification was converted to the 11α-hydroxy derivative (Id). Treatment of 11α-methyl-11β-hydroxyprogesterone (VI)—which was derived from 11α-hydroxyprogesterone (Experimental)—with DDQ yielded a product, m.p. 175–182°, showing a single spot on TLC. The product, according to the IR spectrum and UV absorptions at 250 mμ (ε 14,400) and 300 mμ (ε 1,900), however, appeared to be a mixture of the desired $\Delta^{1,4}$-dien-3-one (If) contaminated with $\Delta^{1,4,6}$-triene-3-one (VIII).[8] Although attempts to purify If failed, the impurity was confirmed as $\Delta^{1,4,6}$-triene-3-one (VIII) by comparison of the IR spectrum with that of pure VIII.

Hydroxylation of the 11α-substituted $\Delta^{1,4}$-3-ones with osmium tetroxide in pyridine was carried out in a manner similar to that used for the 11-oxo compounds. The 11α-hydroxy derivative (Id) yielded the 4β,5β-dihydroxy-Δ^1-3-one (IIId) and small amounts of two other products. The structure IIId for the former was proved by its NMR spectrum exhibiting two olefinic protons at 2·80 and 4·07 τ as two doublets, the β-configuration of the introduced OH groups is discussed later. The two by-products were characterized as the diosphenol (IVd), which was probably formed by dehydration of IIId during isolation, and 4β,5β-dihydroxypregn-1-ene-3,11,20-trione (V), which could result from simultaneous oxidation[9] of the 11α-hydroxy group. In the 11α-hydroxy derivative Id, the addition of osmium tetroxide occurs selectively at the Δ^4 double bond of the $\Delta^{1,4}$-3-oxo system. Osmylation of 11α-acetoxypregna-1,4-diene-3,20-dione (Ie) also affords the 4β,5β-dihydroxy-Δ^1-3-one (IIIe) and its dehydrated diosphenol (IVe). The location of the OH groups in IIIe was established by its NMR spectrum and both IIId and IIIe were chemically correlated by giving the same diosphenol (IVd) on alkali treatment. When 11α-methyl-11β-hydroxypregna-1,4-diene-3,20-dione (If) contaminated with the $\Delta^{1,4,6}$-triene (VIII) as mentioned above was treated with osmium tetroxide, two compounds, m.p. 207–210·5° and m.p. 239–244° (dec) were obtained. The former was established as the 4β,5β-dihydroxy-Δ^1-3-one (IIIf) by elemental analysis and UV, IR and NMR data, and the latter was identified as 6ξ,7ξ-dihydroxy-$\Delta^{1,4}$-3-one (IX), which was prepared by osmylation of the $\Delta^{1,4,6}$-trien-3-one (VIII).

Assignment of the β-configuration for the above 4,5-dihydroxylated products was deduced from earlier results[3] and supported by NMR data. Recently,[7] the effects of C_{11}-substituents upon signals of olefinic C_1-protons in Δ^1-3-oxo and $\Delta^{1,4}$-3-oxosteroids have been investigated, and it has been found that additional shift values of the C_1-proton between 11α-hydroxylated and unsubstituted compounds are −1·15 ppm in 5α-Δ^1-3-oxosteroids and −0·51 ppm in 5β-Δ^1-3-oxo series. When the C_1-proton signal (2·80 τ) of IIId was compared with that (3·24 τ) of 4β,5β-dihydroxypregn-1-ene-3,20-dione,[2,3] the difference (−0·44 ppm) is in good agreement with the value for 5β-Δ^1-3-oxosteroids. In the same way, the values between 11α-acetoxylated and unsubstituted compounds are −0·35 ppm for 5α-Δ^1-3-oxo and +0·28 ppm for 5β-Δ^1-3-oxosteroids. The difference (+0·28 ppm) between the C_1-proton signal (3·52 τ) of IIIe and that (3·24 τ) of the compound having no C_{11}-substituent corresponds with the value of 5β-Δ^1-3-oxosteroids. It is, thus, clear that the addition of osmium

[8] H. J. Ringold and A. Turner, *Chem. & Ind.* 211 (1962).
[9] R. Hirschmann, N. G. Steinberg and R. Walker, *J. Amer. Chem. Soc.* **84**, 1270 (1962).

Id R = H
Ie R = Ac
IIId R = H
IIIe R = Ac
IVd R = H
IVe R = Ac
V
VI
VII
VIII
IX
IIIf
If
DDQ
Chloranil

Chart 3

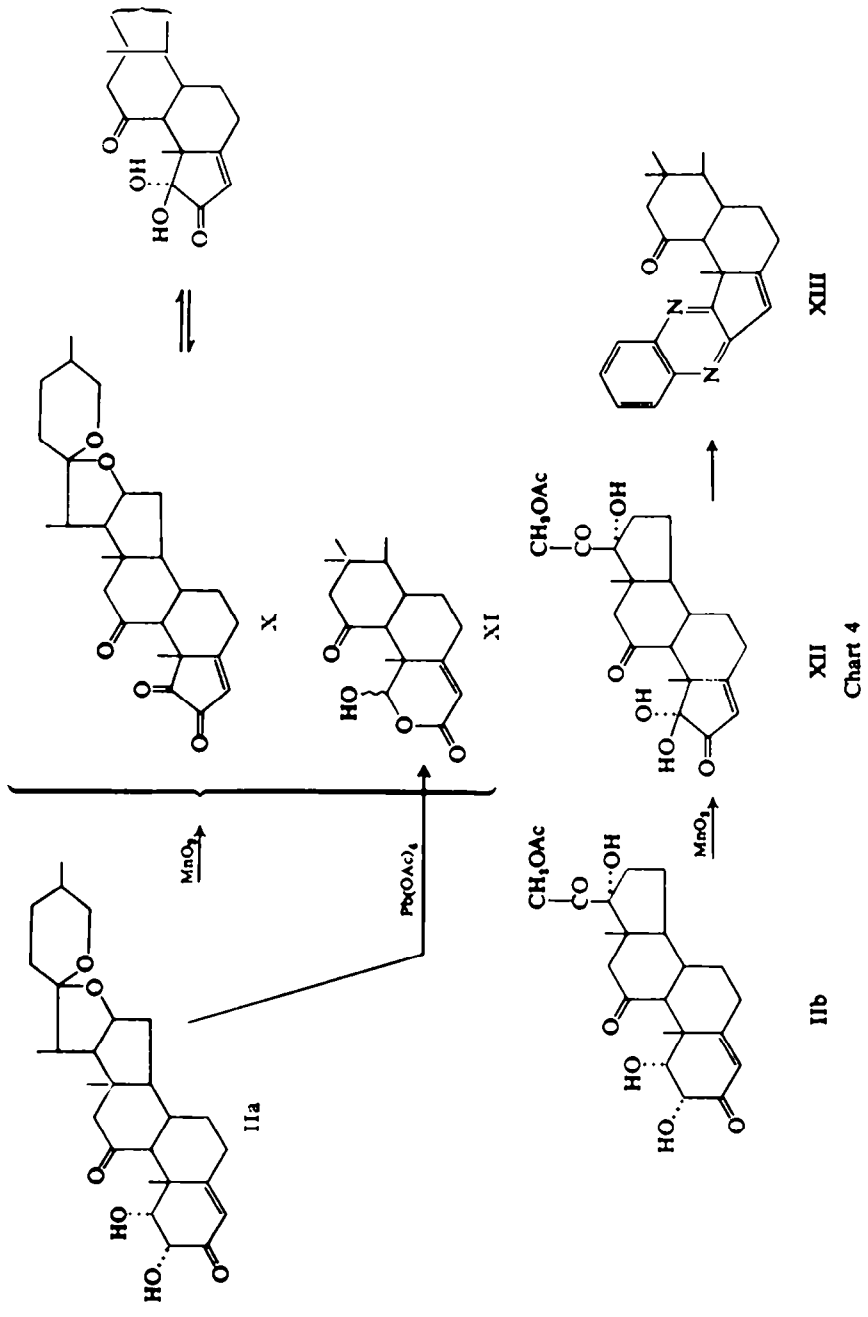


Chart 4

tetroxide to the Δ^4 double bond of the $\Delta^{1,4}$-3-oxosteroids occurs from the β-side of the molecule also when 11α-substituents are present.[3]

The above and previous[2,3] results, have led to the following conclusions. Compounds having no C_{11}-substituent afford nearly equal quantities of 1α,2α-dihydroxy-Δ^4-3-ones and 4β,5β-dihydroxy-Δ^1-3-ones, and the 11β-hydroxy group has no effect on this result. The 11-oxo group, however, results in hydroxylation at the Δ^1 double bond affording exclusively the 1α,2α-dihydroxy-Δ^4-3-ones, while the 11α-substituents such as the 11α-hydroxy, 11α-acetoxy or 11α-methyl-11β-hydroxy group result in the selective addition of osmium tetroxide to the Δ^4 double bond giving the 4β,5β-dihydroxy-Δ^1-3-ones. These abnormal results may be accounted for by the following factors. As suggested recently,[6] the π-electrons of the 11-oxo group probably overlap with those of the Δ^1 double bond in the $\Delta^{1,4}$-3-oxo system. Consequently, the Δ^1 double bond may have an enhanced affinity for osmium tetroxide. On the other hand, the 11α-substituents, which are located close by the Δ^1 double bond, should sterically hinder the approach of osmium tetroxide to the Δ^1 double bond, and would favour the formation of 4β,5β-dihydroxy-Δ^1-3-ones. In addition, the selective addition to the Δ^4 double bond in the 11α-hydroxy derivative Id may be ascribed to diminution of the electron density at the Δ^1 double bond caused by the hydrogen bonding with the 11α-hydroxy group.

The 1α,2α-dihydroxy-Δ^4-3-oxosteroids were obtained only in the 11-oxo series. Of these IIa and IIb were oxidized with manganese dioxide in order to prepare the A-norsteroid derivatives.[2] When progress of the oxidation of IIa was checked by the UV determination, the original absorption at 240 mμ did not change into 280 mμ as expected for the A-nor-$\Delta^{3(5)}$-1,2-dione.[2] Instead, TLC indicated the presence of two new compounds, which were separated by chromatography on silica gel. The first eluted yellow oil crystallized poorly from ether giving yellow crystals, m.p. 221–226°. Its IR and NMR spectra were characteristic of the expected A-nor-$\Delta^{3(5)}$-1,2-dione (X) but the UV spectrum (EtOH) exhibited a weak absorption at 280 mμ (ε 2,300) with a higher intensity at 238 mμ (ε 8,500). Recrystallization of this product from methanol afforded colorless crystals, m.p. 196–208°, the IR spectrum of which showed an OH band and no five-membered carbonyl absorption and, therefore, suggested hydration at the C_1-oxo group. Further elution of the above chromatograph afforded a small amount of a by-product, m.p. 260–267°, which proved to be the lactol XI by identification with a specimen prepared from oxidation of IIa with lead tetraacetate. The 1α,2α-dihydroxy derivative IIb derived from prednisone acetate, on oxidation with manganese dioxide, gave a yellow oil which crystallized poorly from ether giving pale yellow crystals, m.p. 133–136°. The analytical and spectral data suggested that the product was the hydrated form of the expected A-nor-$\Delta^{3(5)}$-1,2-dione (XII), and this was confirmed by formation of the quinoxaline derivative (XIII) by treatment with *o*-phenylenediamine.

It has now been proved that the 1α,2α-dihydroxy-Δ^4-3-oxosteroids having the 11-oxo group can be oxidized with manganese dioxide giving the A-nor-$\Delta^{3(5)}$-1,2-diones as in the absence of substituent at C_{11}. It seems, however, that the A-nor-$\Delta^{3(5)}$-1,2-diones having the 11-oxo group tend to be stabilized by formation of hydrates so as to avoid the dipole repulsion between the 1-oxo and 11-oxo groups. The difficulty in crystallization may be attributed to the existence of an equilibrium mixture between the keto and hydrated forms.

EXPERIMENTAL

All mps determined on a Yanagimoto micro apparatus are uncorrected. Unless otherwise stated, specific rotations were measured in chf solns with Rudolf Photoelectric Polarimeter Model 200. UV spectra were recorded in 95% EtOH solns on a Hitachi EPS-2 recording spectrophotometer. IR spectra were determined with a Nippon Bunko DS-201B spectrometer. NMR spectra were determined at 60 Mc in $CDCl_3$ solns containing TMS as an internal standard using a Varian A-60 analytical NMR spectrometer.

25D-*Spirosta*-1,4-*diene*-3,11-*dione* (Ia)

A mixture of 25D,5α-spirostane-3,11-dione (19 g) and DDQ (34 g) in abs dioxan (950 ml) was refluxed for 23 hr. After cooling, a ppt was removed by filtration. The filtrate was diluted with chf (2 l.) and filtered through a short column of Al_2O_3 (150 g) and the column further eluted with chf. The filtrate and eluate were combined and evaporated giving a crystalline material (13·755 g). Recrystallization from chf–MeOH afforded prisms (6·5 g), m.p. 225–241°, which were recrystallized twice from chf–MeOH and then twice from AcOEt yielding pure Ia, m.p. 248–251°, (lit.[10] m.p. 240–243°). $[\alpha]_D$ +34° (*c*, 0·50). λ_{max} 240 mμ (ε 15,300). (Found: C, 76·55; H, 8·59. Calc. for $C_{27}H_{36}O_4$: C, 76·38; H, 8·55%.)

Androsta-1,4-*diene*-3,11,17-*trione* (Ic)

A soln of androst-4-ene-3,11,17-trione (3 g) and DDQ (3 g) in dry benzene (180 ml) was refluxed for 31 hr. The mixture was cooled and filtered and the filtrate diluted with a half volume of pet. ether was absorbed on a column of Al_2O_3 (60 g). After elution with benzene–pet. ether (2:1), the column was eluted with benzene and with benzene–chf (1:1) yielding crystals (2·015 g), which on recrystallization from MeOH afforded leaflets (1·611 g) of Ic, m.p. 197–198° (lit.[11] m.p. 195–196°). $[\alpha]_D$ +255° (*c*, 0·57). λ_{max} 240 mμ (ε 15,000). (Found: C, 76·37; H, 7·48. Calc. for $C_{19}H_{24}O_3$: C, 76·48; H, 7·43%.)

11α-*Hydroxypregna*-1,4-*diene*-3,20-*dione* (Id) *and its acetate* (Ie)

A soln of 11α-acetoxyprogesterone[12] (4 g) and DDQ (4 g) in anhyd benzene (240 ml) was refluxed for 21 hr. The mixture was filtered and the filtrate was absorbed on a column of Al_2O_3 (100 g) and chromatographed. Elution with benzene–chf (1:1) afforded a crystalline material (3·17 g), which on recrystallization from acetone–n-hexane gave prisms (2·516 g) of Ie, m.p. 176–180°. For analysis, further recrystallization from acetone–n-hexane gave m.p. 181–185°, $[\alpha]_D$ +134° (*c*, 1·13). λ_{max} 245 mμ (ε 16,600). ν_{max}^{Nujol} 1731, 1702, 1670, 1624, 1605, 1234, 883 cm^{-1}. NMR: 3·17 τ (d, J = 11 c/s, C_1—H), 3·85 τ (q, J = 11, 2 c/s, C_2 -H), 3·94 τ (s, C_4—H). (Found: C, 74·69; H, 8·20. $C_{23}H_{30}O_4$ requires: C, 74·56; H, 8·16%.)

The acetate Ie (5 g) was saponified by heating with 1N KOH–MeOH (37 ml) and MeOH (110 ml) under N_2 atm for 30 min. The mixture was neutralized with dil AcOH concentrated to approximately ½ volume and a large volume of H_2O added. The ppt was collected by filtration, washed with H_2O and dried. The product (4·3 g) was recrystallized from aq MeOH giving prisms (2·960 g) of Id, m.p. 223–227°. Further recrystallization from aq MeOH gave an analytical sample, m.p. 225–227·5°, $[\alpha]_D$ +87° (*c*, 1·02), (lit.[13] m.p. 217–218°, $[\alpha]_D$ +89°). λ_{max} 248·5 mμ (ε 18,000). (Found: C, 76·75; H, 8·54. Calc. for $C_{21}H_{28}O_3$: C, 76·79; H, 8·59%.)

11α-*Methyl*-11β-*hydroxypregn*-4-*ene*-3,20-*dione* (VI)

A mixture of 11α-hydroxyprogesterone (19·3 g), *p*-toluenesulphonic acid (1 g), ethylene glycol (40 ml) and dry benzene (400 ml) was refluxed, in a flask fitted with a water separator, for 8 hr. The reaction mixture was washed with 5% $NaHCO_3$ aq and H_2O, dried on Na_2SO_4 and evaporated to

[10] D. N. Kirk, D. K. Patel and V. Petrow, *J. Chem. Soc.* 1046 (1957).

[11] H. L. Hogg, C. C. Payne, M. A. Jevnik, D. Gould, E. L. Shapiro, E. P. Oliveto and E. B. Hershberg, *J. Amer. Chem. Soc.* 77, 4781 (1955).

[12] D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister and H. M. Leigh, *J. Amer. Chem. Soc.* 74, 5933 (1952).

[13] J. Kalvoda, G. Anner, D. Arigoni, K. Heusler, H. Immer, O. Jeger, M. Lj. Mihailovic, K. Schaffner and A. Wettstein, *Helv. Chim. Acta* 44, 186 (1961).

dryness. Recrystallization from acetone containing 1 drop of pyridine afforded needles (11·5 g) of 3,20-*bisethylenedioxypregn-5-en-11α-ol*, m.p. 218–222°. Concentration of the mother liquor gave the second crop (3·24 g), m.p. 210–213°. An analytical sample, m.p. 220–223·5°, was obtained by further recrystallization from MeOH, $[\alpha]_D$ +1° (*c*, 1·10). ν_{max}^{Nujol} 3410, 1093, 1081, 1049, 1040 cm^{-1}. (Found: C, 72·00; H, 9·21. $C_{25}H_{36}O_6$ requires: C, 71·74; H, 9·15%.)

To a soln of the above bisethylene ketal (14·75 g) in pyridine (140 ml), was added a CrO_3–pyridine complex prepared from CrO_3 (10 g) and pyridine (120 ml). The mixture was allowed to stand at room temp overnight, diluted with H_2O (4·5 l.) and extracted thoroughly with AcOEt. The combined extracts were washed with 5% $NaHCO_3$ aq and H_2O, dried over Na_2SO_4 and evaporated to dryness. The product (14·62 g) on recrystallization from acetone–n-hexane gave 3,20-bisethylenedioxypregn-5-en-11-one (10·5 g), m.p. 178–180°, as plates. For analysis, a part was further recrystallized from acetone–n-hexane giving plates, m.p. 181–183°, (lit.[14] m.p. 176–179°). $[\alpha]_D$ +11° (*c*, 0·99). ν_{max}^{Nujol} 1706, 1670, 1098, 1080, 1062, 1055, 1040, 1020 cm^{-1}. (Found: C, 71·96; 8·71. Calc. for $C_{25}H_{36}O_6$: C, 72·08; H, 8·71%.)

A soln of the foregoing 11-one (10·4 g) in dioxan (480 ml) was added dropwise over a period of 15 min to a stirred soln of MeLi in dry ether (185 ml), prepared from Li (2 g) and MeI (20·5 g). The reaction mixture was kept at 65–70° for 5 hr with stirring under N_2 atm and then allowed to stand at room temp overnight. After decomposition of the reagent by addition of H_2O, the mixture was concentrated *in vacuo* until an oil began to separate and extracted with benzene. The organic layer was washed with H_2O, dried over Na_2SO_4 and evaporated to dryness. The residue (10·0 g) on recrystallization from MeOH gave needles (7·40 g), m.p. 182–188°, which showed a weak CO absorption at 1701 cm^{-1} in the IR spectrum. Concentration of the mother liquor afforded an additional crop (1·023 g.)

The combined crystals (8·423 g) were dissolved in acetone (1·2 l.) and *p*-toluenesulphonic acid (1·2 g) was added. The soln was allowed to stand at room temp overnight, neutralized with 5% $NaHCO_3$ aq, diluted with H_2O and concentrated *in vacuo* until a solid separated. The ppt was collected by filtration, washed with H_2O and dried to give the first crop (4·874 g). Extraction of the filtrate with AcOEt and evaporation of the organic layer afforded the second crop (1·542 g). Two recrystallizations of the first crop from aq MeOH gave plates (3·847 g) of VI, m.p. 171–174°, (lit.[15] m.p. 169–172°). $[\alpha]_D$ +180° (*c*, 1·06). λ_{max} 244 mμ (ε 15,600). ν_{max}^{chf} 3605, 3400, 1699, 1667, 1615 cm^{-1}. (Found: C, 75·85; H, 9·43. Calc. for $C_{23}H_{32}O_5 \cdot \frac{1}{2}H_2O$: C, 75·75; H, 9·43%.)

Oxidation of VI *with* DDQ

A soln of VI (3·712 g) and DDQ (3·7 g) in abs benzene (220 ml) was refluxed for 24 hr. The reaction mixture was filtered and the filtrate absorbed on a column of Al_2O_3 (100 g). Elution with benzene–chf (1:1) and with chf gave crystalline fractions (2·649 g), which on recrystallization from aq MeOH afforded crystals (1·90 g), m.p. 115–156°, showing λ_{max} 250, 300 mμ (ε 13,600, 2,420).

The above crystals were chromatographed again on Al_2O_3 (60 g). The top five eluates with benzene–chf (4:1, 200 ml each) were combined (1·180 g) and recrystallization from n-hexane–acetone gave prisms (890 mg), m.p. 175–182°, showing λ_{max} 250, 300 mμ (ε 14,400, 1,900). The product was proved to be a mixture of *11α-methyl-11β-hydroxypregna-1,4-diene-3,20-dione* (If) and VIII by comparison of the IR, UV and NMR spectra with those of pure VIII described below. Attempts to purify If were unsuccessful and the above crystals were subjected to the osmylation reaction. The succeeding fractions of the above chromatography on recrystallization from acetone–n-hexane gave crystals showing higher intensity at 300 mμ (ε 4,280).

General procedure for the hydroxylation of a $\Delta^{1,4}$*-3-oxosteroid*

According to the procedure described,[8] a $\Delta^{1,4}$-3-oxosteroid in pyridine was treated with OsO_4 and the osmate was decomposed with H_2S giving the crude hydroxylated product.

Hydroxylation of Ia *with osmium tetroxide*

A soln of Ia (2·216 g) in pyridine (22 ml) was treated with OsO_4 (1·7 g) in pyridine (9 ml). Working up by the general procedure yielded the crude product (2·2 g) as a foam, which was chromatographed on silica gel (33 g). The fractions (1·50 g) eluted with chf–MeOH (50:1) were crystallized from chf–MeOH giving prisms (744 mg) of *1α,2α-dihydroxy-25D-spirost-4-ene-3,11-dione* (IIa), m.p. 251–257°.

[14] B. J. Magerlein and R. H. Levin, *J. Amer. Chem. Soc.* **75**, 3654 (1953).
[15] Brit. Patent 883,711 (1961).

Concentration of the mother liquor gave additional IIa (286 mg), m.p. 246–256°. Further recrystallization of the first crop from chf–MeOH gave an analytical sample, m.p. 251–256°, $[\alpha]_D$ +42° (*c*, 1·06). λ_{max} 235·5 mμ (ε 12,600). ν_{max}^{Nujol} 3496, 3406, 1707, 1685, 1619 cm^{-1}. NMR: 4·18 τ (s, C_6—H), 5·21 τ (d, J = 3 c/s, C_2—H), 5·59 τ (d, J = 3 c/s, C_1—H), 8·48 τ (s, C_{19}—CH_3), 9·23 τ (s, C_{18}—CH_3). (Found: C, 68·72; H, 8·57. $C_{27}H_{38}O_6 \cdot \frac{1}{2}H_2O$ requires: C, 68·68; H, 8·43%.)

Hydroxylation of prednisone acetate (Ib) *with osmium tetroxide*

A soln of Ib (1·35 g) in pyridine (9 ml) was treated with a soln of OsO_4 (1 g) in pyridine (2 ml). Working up by the general procedure afforded the crude product as a brownish solid (1·21 g), which was chromatographed on silica gel. The earlier fractions (131 mg) eluted with chf–MeOH (200:1) on examination with TLC were recognized as the starting material and after crystallization identified with Ib. The following fractions (105 mg) were recognized as a mixture of the starting material and the 1,2-diol (IIb) described below on TLC determination. The fractions (590 mg) eluted with chf–MeOH (100:1 ~ 50:1) were crystallized from acetone giving needles (208 mg) of 1α, 2α, 17α, 21-*tetrahydroxypregn*-4-*ene*-11,20-*dione* 21-*acetate* (IIb), m.p. 242—247°. Concentration of the mother liquor afforded an additional crop (128 mg) of IIb, m.p. 239–247°. An analytical sample was obtained by further recrystallization of the first crop from acetone. M.p. 244·5–247°, $[\alpha]_D$ +163° (EtOH, *c*, 0·55). λ_{max} 235 mμ (ε 13,200). ν_{max}^{Nujol} 3386, 1755, 1730, 1703, 1670, 1616 cm^{-1}. (Found: C, 63·46; H, 6·96. $C_{25}H_{36}O_8$ requires: C, 63·58; H, 6·96%.)

Hydroxylation of Ic *with osmium tetroxide*

To a soln of Ic (1·441 g) in pyridine (12 ml) was added a soln of OsO_4 (1·44 g) in pyridine (5 ml). The crude product (1·527 g), obtained after working up by the general procedure, was chromatographed on silica gel (16 g). Elution with benzene–chf (1:1) afforded fractions (101 mg), which were shown to be a mixture of the starting material Ic and the 1α,2α-diol IIc described below by TLC and IR comparison. The fractions (727 mg), eluted with chf and with chf–MeOH (100:1), on recrystallization from acetone–pet. ether gave prisms (325 mg) of 1α,2α-*dihydroxyandrost*-4-*ene*-3,11,17-*trione* (IIc), m.p. 201–203°. Concentration of the mother liquor gave an additional crop (154 mg) of IIc, m.p. 185–190°, which on further recrystallization from acetone–pet. ether raised the m.p. to 193–200°. An analytical sample showed m.p. 201–203°, $[\alpha]_D$ +212° (EtOH, *c*, 0·52). λ_{max} 235 mμ (ε 13,200). ν_{max}^{Nujol} 3320, 1726, 1703, 1670, 1616 cm^{-1}. (Found: C, 68·85; H, 7·33. $C_{19}H_{24}O_5$ requires: C, 68·65; H, 7·28%.)

Hydroxylation of Id *with osmium tetroxide*

To a soln of Id (2·96 g) in pyridine (30 ml) was added a soln of OsO_4 (3·0 g) in pyridine (6 ml). The mixture was processed by the general procedure yielding the crude product (2·7 g), which was chromatographed on silica gel. Elution with chf and chf–MeOH (100:1) gave a fraction (488 mg), which showed two spots close together on TLC. This fraction was dissolved in chf and the soln was extracted thoroughly with 2% NaOHaq. The chf soln was washed with H_2O, dried and evaporated leaving a foam (233 mg), which on recrystallization from acetone–n-hexane afforded small needles (73 mg), m.p. 183–216°. Further recrystallization from acetone–n-hexane gave an analytical sample of 4β,5β-*dihydroxypregn*-1-*ene*-3,11,20-*trione* (V), m.p. 215–222°, $[\alpha]_D$ +129° (*c*, 0·99). λ_{max} 228 mμ (ε 8,900). ν_{max}^{chf} 3580, 3488, 1707, 1690, 1618 cm^{-1}. (Found: C, 69·70; H, 7·91. $C_{21}H_{28}O_5$ requires: C, 69·97; H, 7·83%.)

The alkaline layer was acidified with dil AcOH and extracted with chf. The chf extract was washed with H_2O, dried over Na_2SO_4 and evaporated to dryness (126 mg). Crystallization from acetone–n-hexane gave needles (44 mg), which were identical with 4,11α-*dihydroxypregna*-1,4-*diene*-3,20-*dione* (IVd) described below.

Further elution of the above chromatography with chf–MeOH (100:1) afforded fractions (1·366 g), which on crystallization from acetone–n-hexane gave plates (760 mg), m.p. 209–214°. Further recrystallization from acetone–n-hexane gave 4β,5β,11α-*trihydroxypregn*-1-*ene*-3,20-*dione* (IIId) as plates, m.p. 213–215°, $[\alpha]_D$ +106° (*c*, 1·05), λ_{max} 238 mμ (ε 9,450). ν_{max}^{chf} 3580, 3475, 1698, 1681, 1619 cm^{-1}. NMR: 2·80 τ (d, J = 10·5 c/s, C_1—H), 4·07 τ (d, J = 10·5 c/s, C_2—H), 5·42 τ (d, J = 1 c/s, C_4—H), 6·00 τ (m, C_{11}—H), 7·88 τ (s, C_{21}—CH_3), 8·67 τ (s, C_{19}—CH_3), 9·30 τ (s, C_{18}—CH_3). (Found: C, 69·74; H, 8·35. $C_{21}H_{30}O_6$ requires: H, 69·58; H, 8·34%.)

Hydroxylation of Ie *with osmium tetroxide*

To a soln of Ie (3 g) in pyridine (36 ml), OsO_4 (2·7 g) was added. The soln was allowed to stand at room temp for 3 days and the osmate was decomposed by the general procedure described above yielding a foam (2·8 g). Chromatography on silica gel (100 g) was eluted with benzene–chf (1:1) to chf giving a fraction (146 mg), which on recrystallization from acetone–n-hexane afforded plates, m.p. 215–218°, which were identified as 4,11α-*dihydroxypregna*-1,4-*diene*-3,20-*dione* 11α-*monoacetate* (IVe). λ_{max} 245, 301 mμ (ε 9,150, 5,200). ν_{max}^{chf} 3400, 1732, 1705, 1650, 1605 cm^{-1}.

The fractions (1·451 g) eluted further with chf were recrystallized from acetone–n-hexane giving prisms (1·138 g), m.p. 220–225°. Further recrystallization from acetone–n-hexane gave an analytical sample of 4β,5β,11α-*trihydroxypregn*-1-*ene*-3,20-*dione* 11-*monoacetate* (IIIe), m.p. 222–226°, $[\alpha]_D$ +133° (*c*, 1·03). λ_{max} 233 mμ (ε 10,100). ν_{max}^{Nujol} 3514, 3474, 1720, 1704, 1675, 1623 cm^{-1}. NMR: 3·52 τ (d, J = 10·4 c/s, C_1—H), 4·06 τ (d, J = 10·4 c/s, C_2—H), 4·72 τ (m, C_{11}—H), 5·38 τ (d, J = 2 c/s, C_4—H), 7·90 τ (s, 21-CH_3), 7·97 τ (s, acetyl), 8·71 τ (s, 19-CH_3), 9·26 τ (s, 18-CH_3). (Found: C, 68·23; H, 7·98. $C_{23}H_{32}O_6$ requires: C, 68·29; H, 7·97%.)

4,11α-*Dihydroxypregna*-1,4-*diene*-3,20-*dione* (IVd)

(a) *From* IIId. A soln of IIId (30 mg) in MeOH (2 ml) and 1N KOH–MeOH (0·3 ml) was refluxed under N_2 atm for 50 min. The soln was acidified with dil AcOH and evaporated. The residue was taken with chf and the chf soln was washed with H_2O, dried and evaporated giving a residue (15 mg). Two recrystallizations from acetone–n-hexane afforded needles, m.p. 231–234°, which showed no depression on admixture with a sample of IVd obtained in (b). The IR spectra of two samples were superimposable.

(b) *From* IIIe. A soln of IIIe (100 mg) in MeOH (6 ml) and 1N KOH–MeOH (1 ml) was treated as described in (a). Two recrystallizations of the product from acetone–n-hexane afforded an analytical sample of IVd as needles, m.p. 231–235°, $[\alpha]_D$ +120° (*c*, 0·37). λ_{max} 249·5, 300 mμ (ε 9,200, 5,300). ν_{max}^{chf} 3610, 3440, 1702, 1646, 1600, 840 cm^{-1}. NMR: 2·08 τ (d, J = 10·2 c/s, C_1—H), 3·73 τ (d, J = 10·2 c/s, C_2—H), 5·95 τ (m, C_{11}—H), 7·90 τ (s, 21-CH_3), 8·74 τ (s, 19-CH_3), 9·30 τ (s, 18-CH_3). (Found: C, 73·11; H, 8·14. $C_{21}H_{28}O_4$ requires: C, 73·22; H, 8·19%.)

Hydroxylation of If *with osmium tetroxide*

The If contaminated with a small amounts of VIII [λ_{max} 250, 300 mμ (ε 14,400, 1,900)] (500 mg) was treated with OsO_4 (445 mg) in pyridine (6 ml) for 6 days and processed by the general procedure yielding a foam (448 mg). The product was chromatographed on silica gel (20 g). The fractions (280 mg), eluted with chf and chf–MeOH (200:1), were crystallized from acetone–n-hexane giving prisms (197 mg), m.p. 198–207°. Further recrystallization from acetone–n-hexane afforded an analytical sample of 11α-*methyl*-4β,5β,11β-*trihydroxypregn*-1-*ene*-3,20-*dione* (IIIf) as plates, m.p. 207-210·5°, $[\alpha]_D$ +116° (*c*, 1·09). λ_{max} 237·5 mμ (ε 8,200). ν_{max}^{chf} 3580, 3491, 1700, 1680, 1617 cm^{-1}. (Found: C, 70·03; H, 8·76. $C_{23}H_{32}O_6$ requires: C, 70·18; H, 8·57%.)

Further elution of the chromatography with chf–MeOH (50:1) gave another fraction (40 mg), which on recrystallization from MeOH afforded prisms (15 mg), m.p. 217–224° (dec). Recrystallization from acetone–n-hexane gave prisms, m.p. 239–244° (dec), which was characterized as IX and identified with the authentic sample described below. $[\alpha]_D$ +70° (MeOH, *c*, 0·59). λ_{max} 251·5 mμ (ε 14,600). ν_{max}^{Nujol} 3346, 3246, 1687, 1668, 1629, 1601, 890 cm^{-1}. (Found: C, 70·61; H, 8·36. $C_{21}H_{30}O_4$ requires: C, 70·56; H, 8·08%.)

11α-*Methyl*-11β-*hydroxypregna*-4,6-*diene*-3,20-*dione* (VII)

A mixture of VI (500 mg) and chloranil (900 mg) in t-BuOH (35 ml) was refluxed for 3 hr and after cooling filtered. The filtrate and washings were evaporated to leave an oily residue. The residue was dissolved in chf and the soln was washed with H_2O, 5% NaOHaq and H_2O, dried over Na_2SO_4 and evaporated yielding a yellow foam (438 mg). Chromatography on Al_2O_3 (16 g) was eluted with benzene to benzene–chf (4:1) giving a crystalline solid (290 mg), which on recrystallization from aq MeOH afforded prisms (161 mg) of VII, m.p. 207–209·5°. Concentration of the mother liquor gave an additional crop (35 mg), m.p. 203–207°. For analysis, the first crop was recrystallized from aq MeOH, m.p. 207–209·5°, $[\alpha]_D$ +224° (*c*, 1·06). λ_{max} 287 mμ (ε 26,100). ν_{max}^{chf} 3600, 3458, 1703, 1663, 1629, 1584, 878 cm^{-1}. (Found: C, 77·24; H, 8·89. $C_{23}H_{30}O_3$ requires: C, 77·15; H, 8·83%.)

11α-*Methyl*-11β-*hydroxypregna*-1,4,6-*triene*-3,20-*dione* (VIII)

A mixture of VII (175 mg) and DDQ (175 mg) in dry benzene (10 ml) was refluxed for 5 hr. The mixture was filtered and the filtrate was absorbed on a column of Al_2O_3 (4 g). The eluates with benzene–chf (1:1) on evaporation gave an oil (120 mg), which was recrystallized from acetone–n-hexane giving pale yellow prisms, m.p. 108–128°. Further recrystallization from aq MeOH afforded VIII as prisms, m.p. 108–110°, $[\alpha]_D$ +131° (*c*, 1·04). λ_{max} 212·5, 263, 300 mμ (ε 12,500, 10,500, 11,400). ν_{max}^{chf} 3628, 3465, 1701, 1651, 1633, 1603, 1590 cm^{-1}. (Found: C, 75·34; H, 8·73. $C_{22}H_{28}O_3 \cdot \frac{1}{2}H_2O$ requires: C, 75·61; H, 8·37%.)

11α-*Methyl*-6ξ,7ξ,11β-*trihydroxypregna*-1,4-*diene*-3,20-*dione* (IX)

A soln of VIII (77 mg) and OsO_4 (69 mg) in pyridine (1 ml) was allowed to stand at room temp for 3 days. The reaction mixture was processed by the same method as in hydroxylation of a $\Delta^{1,4}$-3-oxosteroid yielding a crystalline solid (72 mg). The product was dissolved in chf and chromatographed on silica gel. The fractions eluted with chf–MeOH (100:1 ~ 50:1) were combined (38 mg) and crystallized from acetone–n-hexane affording prisms (21 mg) of IX, m.p. 240–244° (dec). The IR spectrum was superimposable on that of a specimen of IX described above.

Oxidation of IIa *with manganese dioxide*

To a soln of IIa (900 mg) in chf (90 ml), MnO_2 (9·0 g), prepared by the procedure of Mancera,[16] was added. The mixture was stirred at 32° for 5·5 hr. The MnO_2 was filtered and the filtrate was evaporated giving a yellow foam (530 mg), which was chromatographed on silica gel (16 g). The fractions (390 mg) eluted with chf, on crystallization from ether afforded yellow prisms (45 mg), m.p. 220–223°. Further recrystallization from acetone–n-hexane gave an analytical sample of A-*nor*-25D-*spirost*-3(5)-*ene*-1,2,11-*trione* (X), m.p. 221–226°, $[\alpha]_D$ +54° (*c*, 0·58). λ_{max} 238, 280 mμ (ε 8,540, 2,300). ν_{max}^{chf} 1765, 1721, 1601 cm^{-1}, no OH-band. NMR: 3·42 τ (s, C_9—H), 8·43 τ (s, C_{19}—CH_3), 9·22 τ (s, C_{18}—CH_3). (Found: C, 72·49; H, 7·97. $C_{26}H_{34}O_5 \cdot \frac{1}{2}H_2O$ requires: C, 72·44; H, 8·07%.)

The mother liquor of the above crystallization from ether was evaporated to dryness and the residue on trituration with H_2O gave colorless crystals (160 mg), which were recrystallized from MeOH, m.p. 196–208°. This was characterized as the C_1-hydrate form of X by the following evidences. ν_{max}^{chf} 3560, 3440, 1766 (w), 1723, 1617 (w), 1604 cm^{-1}. (Found: C, 69·98; H, 8·26. $C_{26}H_{34}O_5 \cdot H_2O$ requires: C, 70·24; H, 8·16%.)

The hydrate of X (90 mg) was treated with *o*-phenylenediamine and abs EtOH at refluxing temp for 1·5 hr. After evaporation, the residue was chromatographed on Al_2O_3 (8 g). The eluate (60 mg) with benzene, on crystallization from acetone, gave the *quinoxaline derivative* (41 mg) as plates, m.p. 283–285°. λ_{max} 216·5, 223·5, 260, 337, 355 mμ (ε 23,500, 19,000, 24,000, 12,000, 11,300). ν_{max}^{Nujol} 1725, 1624, 1609, 1584, 1573, 766 cm^{-1}. (Found: C, 76·83; H, 7·84; N, 5·43. $C_{32}H_{36}O_3N_2$ requires: C, 77·07; H, 7·68; N, 5·62%.)

Further elution of the above chromatography yielding X with chf–MeOH (100:1 ~ 50:1) gave a crystalline fraction (45 mg), which on recrystallization from acetone afforded needles (13 mg), m.p. 253–266°. Further recrystallization from acetone gave needles, m.p. 260–267°, which was identical with a sample of 1ξ-*hydroxy*-2-*oxa*-25D-*spirost*-4-*ene*-3,11-*dione* (XI) described below. $[\alpha]_D$ +87° (*c*, 0·12). λ_{max} 224·5 mμ (ε 13,500). ν_{max}^{chf} 3600, 1727, 1704, 1640 cm^{-1}. (Found: C, 70·33; H, 8·26. $C_{26}H_{36}O_6$ requires: C, 70·24; H, 8·16%.)

The above XI (10 mg) was acetylated with Ac_2O (0·15 ml) and pyridine (0·5 ml) by allowing to stand at room temp overnight. The product, extracted with ether by the usual method, was crystallized from acetone yielding the *acetate*, m.p. 254–258°. λ_{max} 225 mμ, ν_{max}^{chf} 1741, 1710, 1648, 1200 cm^{-1}, no OH-band.

1ξ-*Hydroxy*-2-*oxa*-25D-*spirost*-4-*ene*-3,11-*dione* (XI)

To a soln of IIa (27 mg) in benzene (1·5 ml) and MeOH (3 ml), lead tetraacetate (73 mg) was added. After allowing to stand at room temp overnight, the mixture was diluted with benzene (12 ml), washed with H_2O and dried over Na_2SO_4. Evaporation of the benzene soln afforded a residue (27 mg), which was suspended in 50% aq AcOH (0·5 ml) and heated at 100° for 1·5 hr. To the mixture

[16] O. Mancera, G. Rosenkranz and F. Sondheimer, *J. Chem. Soc.* 2189 (1953).

was added H_2O and a ppt was filtered off, washed and dried giving XI (20 mg), m.p. 264–268°. Further recrystallization from acetone gave needles, m.p. 264–270°, which was identified with a sample of XI, obtained as a by-product in MnO_2 oxidation of IIa, by IR spectra and the mixed m.p. determination.

Oxidation of IIb *with manganese dioxide*

A mixture of IIb (340 mg) and MnO_2 (3·4 g) in acetone (34 ml) was stirred at 32° for 4 hr. The mixture was filtered and the filtrate was evaporated leaving a yellow foam (188 mg). The residue was chromatographed on silica gel and elution with chf–MeOH (200:1) afforded a yellow foam (73 mg), which on crystallization from ether gave XII (16 mg) as pale yellow crystals, m.p. 132–136°. For analysis, the crystals were again recrystallized from ether, m.p. 133–136°, λ_{max} 240, 280 mμ (ε 8,300 1,460). ν_{max}^{chf} 3435, 3423, 1751, 1724, 1719, 1693, 1620, 1205 cm^{-1}. (Found: C, 61·86; H, 6·64. $C_{28}H_{36}O_7 \cdot 1\frac{1}{2}H_2O$ requires: C, 62·10; H, 6·87%.)

The mother liquor of crystallization from ether was evaporated and the residue (57 mg) was subjected to the formation of the quinoxaline derivative described below.

The quinoxaline derivative (XIII) *of* XII

The residue (57 mg), obtained from the mother liquor of XII described above, was heated with *o*-phenylenediamine (27 mg) and abs EtOH (12 ml) under refluxing for 2 hr. The soln was evaporated and the residue (84 mg) was purified by a preparative TLC method, using silica gel GF plates and a solvent system of chf–MeOH (9:1). There was obtained the fraction (32 mg) of XIII, which on recrystallization from acetone afforded yellow prisms, m.p. 263–267°. An analytical sample was obtained from further recrystallization from acetone, m.p. 269–272·5°. λ_{max} 216, 223, 259·5, 337, 354 mμ (ε 25,300, 20,700, 25,000, 12,900, 12,000). ν_{max}^{Nujol} 3483, 1729, 1719, 1714, 1622, 1608, 1580, 1574 cm^{-1}. (Found: C, 70·62; H, 6·72. $C_{28}H_{30}O_5N_2$ requires: C, 70·86; H, 6·37%.)

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