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SYNTHETIC STUDIES ON INSECT MOULTING HORMONES, SYNTHESIS OF RUBROSTERONE

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Abstract—A metabolite of insect moulting hormone, rubrosterone was synthesized from dehydroepiandrosterone in 20 steps.

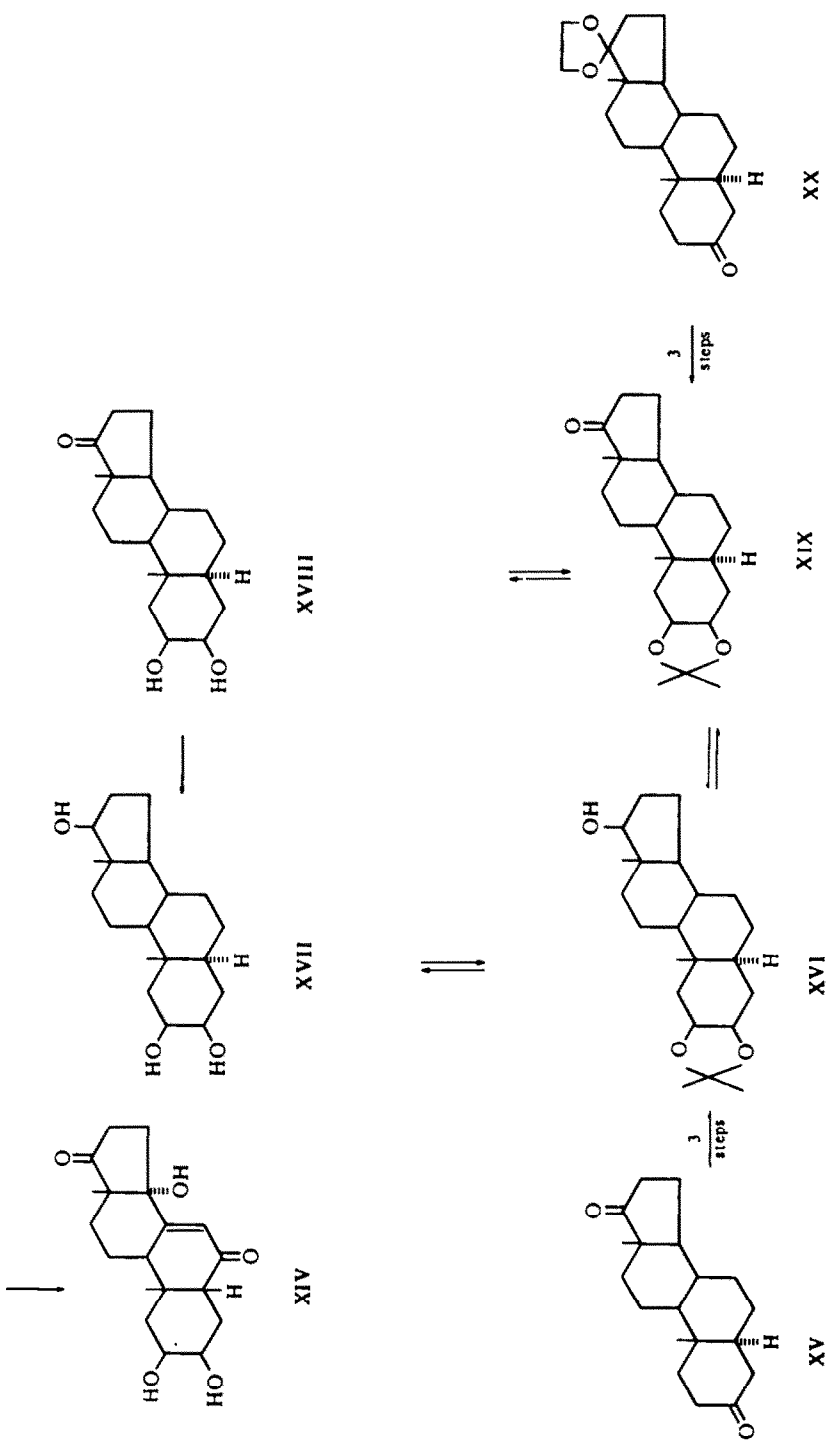
RECENTLY, Takemoto *et al.*[1] isolated a new androstane compound, rubrosterone, from *Achranthes rubrofusca* WIGHT, which is considered to be a metabolite of insect moulting substances and was formulated as 2β,3β,14α-trihydroxy-5β-androst-7-ene-6,17-dione (XIV) on the basis of spectral data. The synthesis of rubrosterone has been achieved by application of the method in our synthesis of ecdysone;[2,3] by the degradation of the side chain of natural insect moulting hormone[4] and by the utilization of the synthetic methods for ecdysone from the Schering and Hoffman-Roche group.[5] The present paper provides a detailed description of the earlier communication[3] of the synthesis of XIV.

3β,17β-Dihydroxy-5α-androstan-6-one (II), chosen as a starting material, was easily prepared from dehydroepiandrosterone (I) in 43% yield by a method similar to that reported.[6] Compound II was transformed into the 6-ketal (III) which was oxidized[7] to the ketone IV in a satisfactory yield.

The 2β,3β-dihydroxy function was constructed by the method reported.[8] The A,B-ring structure of IV is very suitable for this method, but the 17-oxo function would prevent smooth transformation. The reaction rate of autoxidation depends on the enolization of the oxo function. On the other hand, the 3-oxo group is more easily enolized than the 17-oxo group.[9–11] As selective autoxidation, if possible in IV, would provide a convenient synthetic route, 5α-androstane-3,17-dione (XV) was submitted to autoxidation followed by reduction. The treatment of the product with acetone in the presence of phosphomolybdic acid gave an acetonide (XVI). The hydrolysis of XVI afforded a triol, the structure of which was assigned as 5α-androstane-2β,3β,17β-triol (XVII) on the basis of another unambiguous synthesis. 17,17-Ethylenedioxy-5α-androstan-3-one (XX) was submitted to the same procedure to give a diol mixture which, on treatment with acetone and phosphomolybdic acid, gave 2β,3β-isopropylidenedioxy-5α-androstan-17-one (XIX). Reduction of the acetonide (XIX) gave XVI identical with the compound described. All other transformations described in Chart (XVI ⇄ XVII, XVIII ⇄ XIX, XVIII → XVII) support the above structure.

Consequently, these same reactions were applied to IV with satisfactory results. The autoxidation[12] of IV gave the trione as an enol mixture, the IR spectrum of which showed that the D-ring structure (17-oxo function: 1730 cm^{-1}) remained unchanged.

HO
O
I
5 steps
HO
OH
H
O
II
R
R
H
O
O
OH
H
III: R =
IV: R = O
O
O
OH
H
O
V
RO
RO
OR
X
H
O
VI : R = X = H
VII : R = Ac, X = H
VIII : R = Ac, X = Br
AcO
AcO
OAc
H
O
IX
RO
RO
OR
OH
O
X : R = Ac, 5α-H
XIa : R = H, 5α-H
XIb : R = H, 5β-H
O
O
R
OH
O
XIIa: R = OH, 5α-H
XIIb: R = OH, 5β-H
XIII: R = O, 5β-H


XIV
XVII
XVIII
XVI
XIX
XV
XX
3 steps
3 steps

Reduction of the trione followed by treatment of its product with acetone and phosphomolybdic acid afforded the acetonide (V). Mild hydrolysis of V led to the triol (VI),* and the latter was acetylated to the triacetate (VII).

The previous synthetic method for the B,C-ring structure,[13] was applied to the triacetate (VII). Bromination[13,14] of VII afforded the 7α-bromo compound (VIII), the chemical structure of which was confirmed by IR, ORD, and NMR spectra. In the IR spectra of VII and VIII, CO bands appear at 1714 and 1709 cm^{-1}, respectively, and this shows that the Br atom in VIII is axial.[15] The ORD curve of VIII exhibits a negative Cotton effect curve with the same amplitude as that of 7α-bromo-5α-cholestan-6-one,[16] indicative of a A/B-*trans* fusion and a 7α-bromo (axial) structure. Further, the signal due to the 7β-H atom in its NMR spectrum is a singlet (δ 3·35 ppm) resulting from small coupling with a 8β-H (axial) atom.

Compound VIII was dehydrobrominated[17] to the 7-en-6-one (IX) and the latter was converted into an enol acetate.[9] Oxidation of the enol acetate gave the 14α-hydroxy-7-en-6-one (X). NMR spectral data of IX and X are consistent with those of the corresponding compounds in cholestane series.[13]

Hydrolysis of X accompanied by isomerization of C-5 led to an equibrium mixture of XIa and XIb, which was converted into the corresponding acetonide mixture (XIIa and XIIb).* Both acetonides (XIIa and XIIb) were separated in a pure state by TLC. The configuration at C-5 was assigned by R_f value in TLC and the formation ratio;[13] usually, a 5β-acetonide is less polar than a 5α-acetonide in TLC and the formation ratio in a equilibrium mixture is 5β:5α = 4:1. The unambiguous proof was obtained by transformation of XIIb into rubrosterone as described below.

Oxidation[7] of XIIb afforded the 17-oxo compound (XIII), whose IR spectrum is identical with that of the acetonide prepared from natural rubrosterone. XIII was hydrolyzed to rubrosterone (XIV), which was identical with natural rubrosterone by comparison of m.p., optical rotation, and UV, IR, and NMR spectra.[1]

EXPERIMENTAL

All m.ps are uncorrected. Optical rotations were measured in $CHCl_3$ soln unless otherwise stated. IR spectra were recorded on a Hitachi EPI-G2 spectrometer as KBr pellets unless otherwise stated, and UV spectra on a Hitachi EPS-3 spectrometer in EtOH soln. NMR spectra were measured at 60 MHz on a Hitachi H-60 or R-20 spectrometer and at 100 MHz on a Japan Electron Optics 4H-100 spectrometer using $CDCl_3$ as solvent unless otherwise stated. Chemical shifts are given in ppm downfield from internal TMS and coupling constants (J) in Hz. Abbreviations: s = singlet, d = doublet and t = triplet.

6,6-Ethylenedioxy-5α-androstane-3,17-dione (IV)

A mixture of II (5·0 g), *p*-toluenesulfonic acid (0·45 g), ethylene glycol (43·5 ml) and benzene (350 ml) was slowly distilled for 5 hr to half volume. The product was isolated with ether–CH_2Cl_2 to afford III as an oily substance.

A soln of III in pyridine (100 ml) was added at 0° to pyridine–CrO_3 complex prepared from pyridine (100 ml) and CrO_3 (10 g), and the mixture was stirred at room temp for 3 hr and allowed to stand overnight. The product was isolated with ether–CH_2Cl_2 (1:1) and crystallized from MeOH to give IV (3·9 g). An analytical sample was obtained by further crystallization from MeOH as colourless prisms, m.p. 161–163°; $[\alpha]_D^{23}$ +81° (*c*, 1·057); IR ν_{max}: 1735 (C=O at C-17); 1700 cm^{-1} (C=O at C-3). (Found: C, 72·55; H, 8·91. $C_{21}H_{30}O_4$ requires: C, 72·80; H, 8·73%).

* It was fully discussed in the previous paper that these treatments are not accompanied with any isomerization at C-5.

2β,3β-*Isopropylidenedioxy*-17β-*hydroxy*-5α-*androstan*-6-*one* (V)

IV (2·5 g) was added to t-BuOH (100 ml) in which K (3·0 g) was dissolved beforehand, and the suspension was stirred in O_2 atmosphere for 30 min. The resulting soln was poured into ice-water, and acidified to *p*H 2·0 by 10% HCl. The product was isolated with ether–CH_2Cl_2 to give the diosphenol as an oily substance.

To a soln of the product in EtOH (130 ml) was added $NaBH_4$ (0·375 g), and the mixture kept at room temp for 2 hr. After addition of 50% AcOH (50 ml), most of the solvent was distilled off *in vacuo*. The product was isolated with n-BuOH–ether to give the triol mixture as an oily substance.

A soln of the product in Me_2CO (160 ml) and 5% phosphomolybdic acid in Me_2CO (30 ml) was allowed to stand for 30 min and poured into 30% NH_4OH. The product was isolated with ether–CH_2Cl_2 to afford a crystalline residue (1·6 g), which was chromatographed on Florisil. The material eluted with benzene–ether (7:3) was crystallized from Me_2CO–hexane to give V (0·95 g) as colourless needles, m.p. 236–238°; $[\alpha]_D^{23}$ +10° (*c*, 1·185); IR ν_{max} 3240 (OH); 1700 cm^{-1} (C=O). (Found: C, 72·83; H, 9·23. $C_{22}H_{34}O_4$ requires: C, 72·89; H, 9·45%).

2β,3β-*Isopropylidenedioxy*-5α-*androstan*-17β-*ol* (XVI)

(a) *From* XV. XV (0·5 g) was treated as described above to give XVI (0·237 g). Crystallization from Me_2CO–hexane afforded an analytical sample as colourless needles, m.p. 189–193°; $[\alpha]_D^{26}$ +45° (*c*, 0·971; EtOH); IR ν_{max} 3400 cm^{-1} (OH). (Found: C, 75·70; H, 10·72. $C_{22}H_{36}O_3$ requires: C, 75·81; H, 10·41%).

(b) *From* XVII. To a soln of XVII (0·01 g) in Me_2CO (2 ml) was added 5% phosphomolybdic acid in Me_2CO (0·15 ml), and the mixture allowed to stand at room temp for 15 min. 30% NH_4OH was added, and the product was isolated with ether–CH_2Cl_2 to give a crystalline residue, the IR spectrum of which was identical with that of the acetonide obtained above.

(c) *From* XIX. XIX (0·01 g) was reduced with $NaBH_4$ (0·005 g) in MeOH (1 ml), and the soln was poured into H_2O. The product was isolated with ether–CH_2Cl_2 to afford a crystalline material, the IR spectrum of which was identical with that of the acetonide obtained above.

5α-*Androstane*-2β,3β,17β-*triol* (XVII)

(a) *From* XVI. A soln of XVI (0·083 g) in EtOH (20 ml) and 10% H_3PO_4 (4 ml) was refluxed for 1 hr and poured into H_2O. The product was isolated with ether–CH_2Cl_2 and crystallized from Me_2CO–hexane to give XVII (0·061 g, m.p. 220–224°). An analytical sample was obtained by further crystallization from the same solvent as colourless needles, m.p. 226–228°; $[\alpha]_D^{26}$ +36° (*c*, 0·775, EtOH): IR ν_{max}: 3395 cm^{-1} (broad, OH). (Found: C, 74·07; H, 10·38. $C_{19}H_{32}O_3$ requires: C, 73·98; H, 10·46%).

(b) *From* XVIII. XVIII (0·156 g) was treated as in the reduction of XIX. The product was crystallized from Me_2CO–hexane to afford XVII (0·136 g, m.p. 222–226°), which was identical with the material obtained above.

2β,3β-*Isopropylidenedioxy*-5α-*androstan*-17-*one* (XIX)

(a) *From* XX. XX (1·4 g) was treated as described to afford XIX (0·429 g, m.p. 140–165°). An analytical sample was obtained by further crystallization from EtOAc as colourless plates, m.p. 176–180°; $[\alpha]_D^{26}$ +108° (*c*, 0·900; EtOH); IR ν_{max}: 1728 cm^{-1} (C=O). (Found: C, 76·18; H, 9·72. $C_{22}H_{34}O_3$ requires: C, 76·26; H, 9·89%).

(b) *From* XVI. To pyridine–CrO_3 complex prepared from pyridine (0·4 ml) and CrO_3 (0·04 g) was added dropwise a soln of XVI (0·02 g) in pyridine (0·4 ml) at 0°. The mixture was allowed to stand at room temp overnight. The IR spectrum of the product (0·019 g) was identical with that of the acetonide obtained above.

(c) *From* XVIII. XVIII (0·1 g) was treated as in the acetonization of XVII. The product was identical with the acetonide obtained above.

2β,3β-*Dihydroxy*-5α-*androstan*-17-*one* (XVIII)

Compound XIX (0·48 g) was treated as described to give XVIII (0·412 g, m.p. 182·5–184·5°). An analytical sample was obtained by further crystallization from the same solvent as colourless needles, m.p. 183–184·5°; $[\alpha]_D^{26}$ +107° (*c* 1·20; EtOH); IR ν_{max} 3405 (OH); 1730 cm^{-1} (C=O). (Found: C, 74·42; H, 9·55. $C_{19}H_{30}O_3$ requires: C, 74·47; H, 9·87%).

2β,3β,17β-*Trihydroxy*-5α-*androstan*-6-*one* (VI)

Compound V (0·4 g) was treated as described to afford VI (0·247 g, m.p. 232–238°). An analytical sample was obtained by further crystallization from Me_2CO as colourless prisms, m.p. 237–240°; $[\alpha]_D^{23}$ −6°

(*c.* 1·24; MeOH); IR ν_{max}: 3320 (broad. OH); 1698 cm^{-1} (C=O). (Found: C. 70·56; H. 8·93. $C_{19}H_{30}O_4$ requires: C. 70·77; H. 9·38%).

2β.3β.17β-*Triacetoxy-5α-androstan*-6-one (VII)

A soln of VI (0·248 g) in Ac_2O (10 ml) was refluxed for 2 hr and poured into H_2O. The product was isolated with ether. and crystallized from MeOH to give VII (0·299 g. m.p. 209–210°). An analytical sample was obtained by further crystallization from MeOH as colourless needles. m.p. 210–212°; $[\alpha]_D^{23}$ −10° (*c.* 1·510); IR $\nu_{max}^{CCl_4}$: 1740 (OAc); 1714 cm^{-1} (C=O). (Found: C. 66·94; H. 8·07. $C_{25}H_{36}O_7$ requires: C. 66·94; H. 8·09%).

2β.3β.17β-*Triacetoxy-7α-bromo-5α-androstan-6-one* (VIII)

To a soln of VII (0·618 g) in AcOH (6 ml) containing a trace of HBr was added a soln of Br_2 (0·219 g) in AcOH (1·4 ml). The soln was warmed at 50° with stirring for 2 hr and poured into H_2O. The product was isolated with ether and crystallized from ether–hexane to give VIII (0·596 g. m.p. 212–215°. dec). An analytical sample was obtained by further crystallization from MeOH as colourless prisms. m.p. 224–225°. dec; $[\alpha]_D^{23}$ +48° (*c.* 0·600); IR $\nu_{max}^{CCl_4}$: 1733 (OAc); 1709 cm^{-1} (C=O). (Found: C. 56·92; H. 6·72. $C_{25}H_{35}O_7Br$ requires: C. 56·88; H. 6·75%).

2β.3β.17β-*Triacetoxy-5α-androst-7-en-6-one* (IX).

Compound VIII (0·281 g) was heated under reflux with DMF (5·6 ml) and Li_2CO_3 (0·28 g) for 2 hr in N_2 atmosphere. After addition of 10% HCl. the product was isolated with ether and crystallized from ether–hexane to give IX (0·619 g. m.p. 225–228°). An analytical sample was obtained by further crystallization from MeOH as colourless prisms. m.p. 228–230°; $[\alpha]_D^{23}$ −5° (*c.* 0·561); IR $\nu_{max}^{CHCl_3}$: 1731 (OAc); 1672 (C=O); 1620 cm^{-1} (C=C); NMR: 0·70 (18-CH_3. 3H. s); 1·00 (19-CH_3. 3H. s); 5·75 (7-H. 1H. t. J = 2·5); 2·02 (OAc. 3H. s); 2·05 (OAc. 3H. s); 2·08 (OAc. 3H. s); UV λ_{max}: 242 mμ (ε. 14.000). (Found: C. 67·50; H. 7·57. $C_{25}H_{34}O_7$ requires: C. 67·24; H. 7·68%).

2β.3β.17β-*Triacetoxy*-14α-*hydroxy-5α-androst-7-en-6-one* (X)

Compound IX (0·34 g) was dissolved in 0·001M $HClO_4$ in EtOAc (31·7 ml) and Ac_2O (3·4 ml). and the soln was allowed to stand at room temp for 15 min. The mixture was poured into 5% $KHCO_3$ aq and the product isolated with ether to give the enol acetate as an oily substance; IR $\nu_{max}^{CS_2}$: 1750 (OAc at C-6); 1740 (OAc at C-2, C-3 and C-17); UV λ_{max}: 253 mμ.

The enol acetate was dissolved in anhyd ether (34 ml). and a soln of perphthalic acid (0·179 g) in anhyd ether (1·2 ml) added. The mixture was allowed to stand at room temp overnight. The soln was washed with 4% NaOH aq and H_2O. and dried (Na_2SO_4). Removal of the solvent followed by crystallization from ether gave X (0·209 g. m.p. 237–240°). An analytical sample was obtained by further crystallization from ether as colourless prisms. m.p. 237–240°; $[\alpha]_D^{23}$ +49° (*c.* 1·666); IR ν_{max}: 3455 (OH); 1668 (C=O); 1620 cm^{-1} (C=C); NMR: 0·75 (18-CH_3. 3H. s); 0·99 (19-CH_3. 3H. s); 2·02 (OAc. 3H. s); 2·05 (OAc. 3H. s); 2·08 (OAc. 3H. s); 5·89 (7-H. 1H. d. J = 2·5); UV λ_{max}: 239 mμ (ε. 11.000). (Found: C. 64·66; H. 7·41. $C_{25}H_{34}O_8$ requires: C. 64·92; H. 7·41%).

14α,17β-*Dihydroxy*-2β,3β-*isopropyridenedioxy-5α-androst-7-en-6-one* (XIIa) *and* 14α,17β-*Dihydroxy*-2β,3β-*isopropylidenedioxy-5β-androst-7-en-6-one* (XIIb)

A soln of X (0·395 g) in 0·6% K_2CO_3 in 90% aqueous MeOH (79·5 ml) was allowed to stand at room temp for 1 hr and then refluxed for 30 min. The soln was poured into H_2O. and the product was isolated with n-BuOH–ether to afford XIa and XIb as an oily mixture.

This tetrol mixture was dissolved in Me_2CO (31 ml). and a soln of 5% phosphomolybdic acid in Me_2CO (5·9 ml) was added. The mixture was allowed to stand at room temp for 20 min. and the soln was poured into 30% NH_4OH. The product was isolated with ether–CH_2Cl_2. and submitted to the preparative TLC on silica gel. Merck GF_{254} (0·5 mm plates. developing solvent $CHCl_3$–EtOH–Me_2CO (70:2·5:30)). The material obtained from the polar fraction was crystallized from Me_2CO–hexane to yield XIIa (0·023 g) as colourless prisms. m.p. 280–285°; $[\alpha]_D^{23}$ +7° (*c.* 0·222; MeOH); IR ν_{max}: 3420 (OH); 1660 cm^{-1} (C=O); UV λ_{max}: 243 mμ (ε. 9.900). (Found: C. 68·68; H. 8·40. $C_{22}H_{32}O_5 \cdot \frac{1}{2}H_2O$ requires: C. 68·54; H. 8·63%).

The material from the nonpolar fraction was crystallized from Me_2CO to yield XIIb (0·102 g) as colourless needles. m.p. 250–256°; $[\alpha]_D^{23}$ +24° (*c.* 0·610; MeOH); IR ν_{max}: 3350 (OH); 1640 cm^{-1} (C=O); UV λ_{max}: 240 mμ (ε. 10.900). (Found: C. 68·39; H. 8·71. $C_{22}H_{32}O_5 \cdot \frac{1}{2}H_2O$ requires: C. 68·54; H. 8·63%).

14α-Hydroxy-2β.3β-isopropylidenedioxy-5β-androst-7-ene-6.17-dione (XIII)

A soln of XIIb (0·04 g) in pyridine (1·6 ml) was added to pyridine–CrO_3 prepared from pyridine (1·6 ml) and CrO_3 (0·16 g) at 0°, and the mixture was allowed to stand at room temp overnight. The product was isolated with ether–CH_2Cl_2, and crystallized from ether–hexane to give XIII (0·032 g) as colourless needles, m.p. 233–237°; $[\alpha]_D^{23}$ +102° (*c*, 0·363; MeOH); IR ν_{max}: 3390 (OH); 1729 (C=O); 1673 cm^{-1} (C=O); UV λ_{max}: 239 mμ (ε, 11.000). (Found: C, 70·51; H, 7·81. $C_{22}H_{30}O_5$ requires: C, 70·56; H, 8·08%).

2β.3β.14α-Trihydroxy-5β-androst-7-ene-6.17-dione (Rubrosterone) (XIV)

Compound XIII (0·02 g) was dissolved in a soln of 0·1N HCl in 90% aqueous THF (0·7 ml), and the mixture was allowed to stand at room temp for 4 hr. After addition of H_2O, the product was isolated with n-BuOH–ether to yield a crystalline material, which on crystallization from Me_2CO–ether gave rubrosterone identical with the natural product in IR spectrum, m.p. 240–244°, dec; $[\alpha]_D^{23}$ +125° (*c*, 0·216, MeOH); IR ν_{max}: 3445 (broad, OH); 1744 (C=O); 1648 cm^{-1} (C=O); NMR (C_5D_5N): 0·85 (18-CH_3, 3H, s); 1·03 (19-CH_3, 3H, s); 6·24 (7-H, 1H, d, J = 2·5); UV λ_{max}: 239 mμ (ε, 10.300).

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