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133. Issei Iwai and Tetsuo Hiraoka : Studies on Acetylenic Compounds. XV.*2 Synthesis of 16-Ethynylated Steroids.

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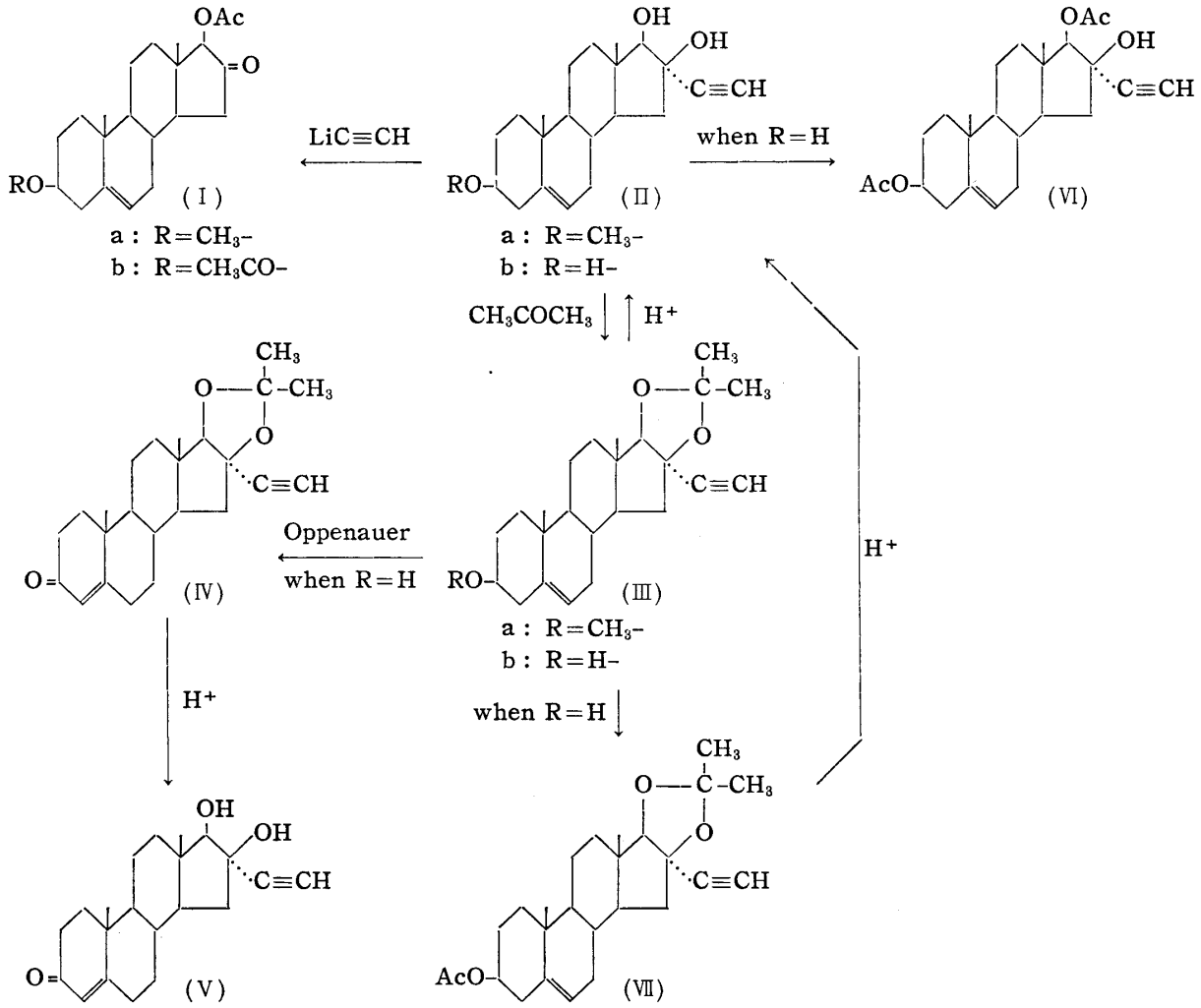
Recently, many works have been reported on alkylated steroids, some of which showed marked increase of biological activity compared to the parent compounds. With respect to methylation at the 16-position in glucocorticoidal steroid series,[1] pregnane series,[2] and androstane series,[3] many reports have been published and glucocorticoidal steroids with methyl group at the 16-position are commercially available for medical use.



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Considering that 17-ethynylated steroid is more important in variety of its action, attempts were made to synthesize 16-ethynylated androstene derivatives. This paper is a part of a recent communication[4] concerning ethynylated steroids.

3β-Methoxy-17β-acetoxyandrost-5-en-16-one (Ia) and 3β,17β-diacetoxyandrost-5-en-16-one (Ib) prepared from dehydroepiandrosterone according to the method of Stodola and Huffman[5] were ethynylated with lithium acetylide in liquid ammonia. The conversion of (Ia) to 3β-methoxy-16α-ethynylandrost-5-ene-16β,17β-diol (IIa), m.p. 194~196°, $[\alpha]_D^{22}$ −66°($CHCl_3$), proceeded in relatively good yield but the same reaction of (Ib) to 16α-ethynylandrost-5-ene-3β,16β,17β-triol (IIb), m.p. 251~253°, $[\alpha]_D^{24.5}$ −65.8°($CHCl_3$), did not proceed in better yield. The isolation of (IIb) in pure state was difficult and it was submitted to the next reaction to give 16α-ethynyl-16,17-O-isopropylideneandrost-5-ene-3β,16β,17β-triol (acetonide) (IIIb), m.p. 162~165°, $[\alpha]_D^{26}$ −16.2°($CHCl_3$), without purification. On the isolation of (IIIb) from the reaction mixture, a brown solid substance, sparingly soluble in ether but easily soluble in alcohol, was obtained. This substance was acetylated and subsequent purification of the acetate on alumina afforded 16α-ethynylandrost-5-ene-3β,16β,17β-triol 3,17-diacetate (VI), m.p. 201~203°, $[\alpha]_D^{24.5}$ −66.7°($CHCl_3$), and an unknown substance. The latter was not an isomer of (VI) at 16-position, since its infrared spectrum showed ≡C-H stretching band but no hydroxyl. The elemental analysis also did not agree with an isomer of (VI) and the ultraviolet spectrum showed only end absorption in the region of 220~340 mμ. Further investigation was not carried out.

When (Ib) was submitted to ethynylation according to the method of Stavely[6] with introduction of acetylene gas in the presence of potassium *tert*-pentyloxide into the ether solution, a new substance was obtained which unexpectedly had no ethynyl group and which showed negative α-ketol test. Sondheimer, *et al.*[7] reported that they had similar result in ethynylation of androsta-1,4-diene-3,17-dione at the 17-position. Treating of (IIa) and (IIb) with acetone containing dry hydrogen chloride afforded 16,17-acetonide. This result proves that 16-hydroxyl substituent is *cis* to 17-hydroxyl. As the configuration of 17-hydroxyl group has been known to be β-oriented,[8] 16-hydroxyl group is also β-oriented and therefore the attack of lithium acetylide to (I) proceeded from α-side in the steroid molecule as usual. Oppenauer oxidation of 16α-ethynyl-16,17-O-isopropylideneandrost-5-ene-3β,16β,17β-triol (IIIb) gave 16α-ethynyl-16β,17β-isopropylidenedioxyandrost-4-en-3-one (IV), m.p. 156~157°, $[\alpha]_D^{25.1}$ +83.6°($CHCl_3$), which was converted into 16α-ethynyl-16β,17β-dihydroxyandrost-4-en-3-one (V), m.p. 208~211°, $[\alpha]_D^{28}$ +88.3°($CHCl_3$), by hydrolysis in ethanol-water in the presence of hydrochloric acid at room temperature.

Experimental

All m.p.s are uncorrected. Rotations were determined in $CHCl_3$ solution, unless otherwise stated.

3β-Methoxy-16α-ethynylandrost-5-ene-16β,17β-diol (IIa) and 3β-Methoxy-16α-ethynyl-16,17-O-isopropylideneandrost-5-ene-16β,17β-diol (IIIa)—Dried acetylene gas was bubbled into 70 cc. of liquid NH_3 for 30 min., 170 mg. of Li was added to the solution in small pieces. Acetylene gas was further introduced for 1 hr., then 500 mg. of 16-oxosteroid (Ia) in 30 cc. of dehyd. Et_2O and 5 cc. of dehyd. benzene was added. This reaction mixture was stirred for 4 hr. at −45° with continuous introduction of acetylene gas. After allowing to stand overnight under cooling by dry ice-acetone bath,

4) This Bulletin, **7**, 394(1959) (communication).
5) F. H. Stodola, *et al.* : J. Org. Chem., **6**, 841(1941); M. N. Huffman, *et al.* : J. Biol. Chem., **172**, 789(1948).
6) H. E. Stavely : J. Am. Chem. Soc., **61**, 79(1939).
7) O. Mancera, G. Rosenkranz, F. Sondheimer : *Ibid.*, **77**, 5673(1955).
8) M. N. Huffman, *et al.* : J. Am. Chem. Soc., **71**, 719(1949). It should be noted that the orientation of the D-ring hydroxyl groups reported in this paper is the reverse of that currently accepted. See J. Am. Chem. Soc., **76**, 2943(1954) footnote (9).

liquid NH_3 was allowed to evaporate at room temperature under continuous mechanical stirring. A solution of 4 g. of NH_4Cl in 15 cc. of H_2O was added, then the mixture was poured into cold 3% H_2SO_4, the organic layer was separated, and the aqueous layer was extracted with Et_2O. The combined organic extract was washed with aqueous $NaHCO_3$ solution and H_2O until neutral to litmus, dried over Na_2SO_4, and evaporated under reduced pressure. The gummy residue (461 mg.) crystallized on treatment with benzene. Recrystallization from benzene gave 3β-methoxy-16α-ethynylandrost-5-ene-16β,17β-diol (IIa) of m.p. 190~196°. Further two recrystallization from MeOH gave prisms of m.p. 194~196° with previous softening, weighing 43 mg. *Anal.* Calcd. for $C_{22}H_{32}O_3$: C, 76.70; H, 9.36; O, 13.93. Found : C, 76.95; H, 9.08; O, 13.84. $[\alpha]_D^{22}$ −66°, IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3340 (≡CH), 3570 (OH).

All mother liquors were collected and evaporated to dryness under reduced pressure. The residue (383 mg.) was dissolved in 30 cc. of dehyd. Me_2CO, 5 cc. of Me_2CO saturated with dry HCl was added, this reaction mixture was allowed to stand at room temperature for 2.5 hr. and poured into 300 cc. of ice-cooled 3% Na_2CO_3 solution. The Et_2O extracts were washed with H_2O until neutral to litmus, dried, and evaporated to dryness. Recrystallization of the yellow solid residue (475 mg.) from MeOH, gave 3β-methoxy-16α-ethynyl-16,17-O-isopropylideneandrost-5-ene-16β,17β-diol (IIIa) as needles of m.p. 149~162° with previous softening. Further recrystallization from the same solvent gave m.p. 156~159° (at 162° became clear), weighed 80 mg. The mother liquors obtained above was evaporated to dryness under a reduced pressure. The brown gummy residue (260 mg.) was chromatographed over 15 g. of neutral alumina. Recrystallization of the amorphous substance eluted with petr. ether-benzene (1:2) from MeOH gave 3β-methoxy-16α-ethynyl-16,17-O-isopropylideneandrost-5-ene-16β,17β-diol (IIIa) as needles of m.p. 159~161° (at 163° becoming clear), weighed 31 mg. *Anal.* Calcd. for $C_{25}H_{36}O_3$: C, 78.08; H, 9.44. Found : C, 78.08; H, 9.39. $[\alpha]_D^{23.3}$ −18.1°.

Hydrolysis of (IIIa) gave (IIa) which was identified as (IIa) by admixture with the authentic sample.

16α-Ethynylandrost-5-ene-3β,16β,17β-triol (IIb) and 16α-Ethynyl-16,17-O-isopropylideneandrost-5-ene-3β,16β,17β-triol (IIIb)–Dry acetylene gas was bubbled through into 800 cc. of liquid NH_3 for 1 hr., then 4.8 g. of Li was added to this solution in small portions at such a rate that the blue color vanished within 2 min. After introduction of acetylene gas for 1 hr., 15 g. of the 16-oxosteroid (Ib) in 900 cc. of dehyd. Et_2O was added during 1.5 hr. and this reaction mixture was stirred for 8.5 hr. at −45°. After keeping overnight at −70°, liquid NH_3 was evaporated at room temperature with continuous stirring. 74 g. of NH_4Cl was added under cooling with ice-water and H_2O was added. The solution was poured into 5% H_2SO_4 (containing ice), the organic layer was separated, and the aqueous layer was extracted with AcOEt. The combined organic extracts were washed with $NaHCO_3$ solution and H_2O, dried, and evaporated under reduced pressure. Treatment of the residue (12.2 g.) with benzene gave amorphous solid which on recrystallization repeatedly from Me_2CO gave 16α-ethynylandrost-5-ene-3β,16β,17β-triol (IIb) as needles of m.p. 251~253° with previous softening. *Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found : C, 76.56; H, 9.04. $[\alpha]_D^{24.5}$ −65.8°, IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3310 (≡C-H), 3550 (OH).

The crude substance obtained from the mother liquor (about 12 g.) was dissolved in 210 cc. of dehyd. Me_2CO to which 30 cc. of Me_2CO saturated with dry HCl was added and this reaction mixture was allowed to stand at room temperature for 2 hr. This was poured into 2,000 cc. of 3% Na_2CO_3 solution (containing ice) and extracted with Et_2O. The extract was washed with H_2O until neutral to litmus, dried, and evaporated to dryness. The residue was a mixture of oil and solid, and weighed 9.8 g. This was dissolved in 300 cc. of dehyd. Et_2O and adsorbed on 300 g. of neutral alumina. Elution with Et_2O gave 3.4 g. of 16α-ethynyl-16,17-O-isopropylideneandrost-5-ene-3β,16β,17β-triol (IIIb) which melted at 141~149°. Recrystallization from hexane gave needles, m.p. 162~165°. *Anal.* Calcd. for $C_{24}H_{34}O_3$: C, 77.80; H, 9.25. Found : C, 77.90; H, 9.18. $[\alpha]_D^{26}$ −16.2°.

On extraction of the ethynylating reaction mixture with Et_2O and AcOEt, insoluble substance remained which was collected and dissolved in EtOH to which AcOEt was added. This organic solution was washed with NaCl solution until neutral to litmus, dried, and evaporated to dryness under reduced pressure. The brown oily residue (3.3 g.) was acetylated with 45 cc. of pyridine and 15 cc. of Ac_2O. The usual processing gave oily substance which was chromatographed over 100 g. of alumina. The first fraction eluted with benzene gave 1.27 g. of solid which on crystallization from EtOH gave flakes of m.p. 211~213°. Its IR spectrum showed the presence of -C≡C-H but no hydroxyl band. Further investigations on this substance were not conducted.

The second fraction eluted with benzene gave 16α-ethynylandrost-5-ene-3β,16β,17β-triol 3,17-diacetate (VI) of m.p. 199~203° which showed no depression of the m.p. on admixture with the authentic sample. *Anal.* Calcd. for $C_{25}H_{34}O_5$: C, 72.43; H, 8.27; O, 19.30. Found : C, 72.27; H, 8.30; O, 18.96. $[\alpha]_D^{24.5}$ −66.7°.

16α-Ethynyl-16β,17β-isopropylidenedioxyandrost-4-en-3-one (IV)—1.95 g. of (IIIb) was suspended in 40 cc. of toluene and 10 cc. of cyclohexanone, and 5 cc. of the mixed solvents was distilled off to remove moisture. To this solution, 600 mg. of Al(iso-PrO)$_3$ in 10 cc. of toluene was added, and 12 cc. of distillate was collected during this time. After distilling off further 6 cc. of toluene, the

reaction mixture was refluxed for 1 hr. To the cooled solution, Et_2O and saturated aqueous solution of potassium sodium tartarate were added and the mixture was shaken in a separatory funnel. After the precipitate was filtered off, the organic solvent was washed with H_2O, dried, and evaporated to dryness under reduced pressure. Recrystallization of the residue from hexane gave 16α-ethynyl-16β,17β-isopropylidenedioxyandrost-4-en-3-one (IV) of plates, m.p. 135~146°, weighed 1.26 g. Further recrystallization from hexane-EtOH gave plates of m.p. 156~157° (575 mg.).

The crude substance obtained from the mother liquors was purified by alumina-chromatography to give a pure substance of m.p. 155~156° (630 mg.), $[\alpha]_D^{25.1}$ +83.6°. *Anal.* Calcd. for $C_{24}H_{32}O_3$: C, 78.22; H, 8.75. Found: C, 78.26; H, 8.63. IR $\nu_{max}^{C_6H_{14}}$ cm^{-1}: 3320 (≡C-H), 1679 (α,β-unsaturated ketone). UV λ_{max}^{EtOH} mμ (log ε): 238.5 (4.20).

16α-Ethynyl-16β,17β-dihydroxyandrost-4-en-3-one (V)—A mixed solution of 40 cc. of 10% HCl and 40 cc. of EtOH was added to a solution of 1.1 g. of (IV) dissolved in 40 cc. EtOH. This reaction mixture was allowed to stand at room temperature for a night, poured into 80 cc. of water, and cooled in a refrigerator for a night. After filtration, the crystalline substance was dried (846 mg.). Recrystallization from hydrous EtOH gave 16α-ethynyl-16β,17β-dihydroxyandrost-4-en-3-one (V) of needles of m.p. 198~203°, weighed 818 mg. Further recrystallization from hydrous EtOH gave m.p. 208~211° with previous softening. *Anal.* Calcd. for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found: C, 76.35; H, 9.05. $[\alpha]_D^{28}$ +88.3. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3320 (≡C-H), 1669 (α,β-unsaturated ketone), 3400 (OH).

16α-Ethynylandrost-5-ene-3β,16β,17β-triol 3,17-Diacetate (VI)—Fifty milligrams of 16α-ethynylandrost-5-ene-3β,16β,17β-triol was acetylated with 10 cc. of pyridine and 3 cc. of Ac_2O. The usual processing and recrystallization from EtOH gave 45 mg. of 16α-ethynylandrost-5-ene-3β,16β,17β-triol 3,17-diacetate (VI) as fine plates of m.p. 201~203°. *Anal.* Calcd. for $C_{25}H_{34}O_5$: C, 72.43; H, 8.27; O, 19.30. Found: C, 72.27; H, 8.30; O, 18.96. $[\alpha]_D^{24.5}$ −66.7°. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3310 (≡C-OH), 3580 (OH), 1750 (Acetyl).

16α-Ethynyl-16,17-O-isopropylideneandrost-5-ene-3β,16β,17β-triol 3-Acetate (acetonide) (VII)—1.9 g. of 16α-ethynyl-16,17-O-isopropylideneandrost-5-ene-3β,16β,17β-triol was acetylated with 15 cc. of pyridine and 5 cc. of Ac_2O at room temperature as usual. The usual processing and recrystallization from hexane gave 1.8 g. of 16α-ethynyl-16,17-O-isopropylideneandrost-5-ene-3β,16β,17β-triol 3-acetate (VII) of fine plates of m.p. 140~142°. Further recrystallization from the same solvent gave white plates of m.p. 141~142.5°. $[\alpha]_D^{28.5}$ −22.5°. *Anal.* Calcd. for $C_{26}H_{36}O_4$: C, 75.69; H, 8.80; O, 15.51. Found: C, 76.16; H, 8.85; O, 15.24.

16α-Ethynylandrost-5-ene-3β,16β,17β-triol (IIb) by Hydrolysis of 16α-Ethynyl-16,17-O-isopropylideneandrosta-3β,16β,17β-triol 3-Acetate (VII)—To a solution of 950 mg. of 16α-ethynyl-16,17-O-isopropylideneandrost-5-ene-3β,16β,17β-triol 3-acetate in 45 cc. of EtOH, a mixture of 17 cc. of 10% HCl and 14 cc. of EtOH was added. On this addition, the steroid reappeared in fine needles, but this was allowed to stand at room temperature for a night after addition of 4 cc. of EtOH. After standing overnight, the solution became clear, to which a mixture of 17 cc. of 10% HCl and 14 cc. of EtOH was added. After 4 hr. the reaction mixture was poured into 700 cc. of H_2O and the precipitate was filtered, washed with H_2O until neutral to litmus. Recrystallization from hydr. EtOH gave 542 mg. of 16α-ethynylandrost-5-ene-3β,16β,17β-triol (IIb) which melted at 220~225° (became clear at 233°). Three recrystallizations from Me_2CO gave a sample of m.p. 251~253°, $[\alpha]_D^{24.5}$ −65.8° (dioxane), no depression in m.p. was observed on admixture with the authentic sample.

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Summary

3β,17β-Diacetoxyandrost-5-en-16-one and 3β-methoxy-17β-acetoxyandrost-5-en-16-one were ethynylated with lithium acetylide. The resulting 16α-ethynyl-16β,17β-diol afforded 16,17-acetonide on treating with acetone, one of which was converted into 16α-ethynyl-16β,17β-dihydroxyandrost-4-en-3-one by Oppenauer oxidation of the corresponding 3-ol and hydrolysis of 16,17-acetonide.

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