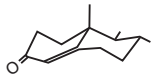
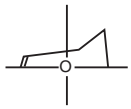
CD spectra of pharmaceutical substances - Steroids (1)

1. Testosterone

Testosterone is a typical male hormone although it is not described in the Japanese Pharmacopoeia. The synthesis and secretion of testosterone in the testes is accelerated by gonadotrophic hormones in the pituitary and the blood concentration of testosterone is kept constant by a feedback mechanism involving the pituitary. (Average blood concentration for humans: 0.64 µg/dl for male and 0.034 µg/dl for female).[1] In the cells of the prostate, one of the targeted organs, testosterone is reduced to 5α-dihydrotestosterone (stanolone), shown in Figure 2, then binds with an androgen-receptor, enters into a nucleus and finally functions to activate a gene.[1]



The half-chair type conformation of the A-ring.

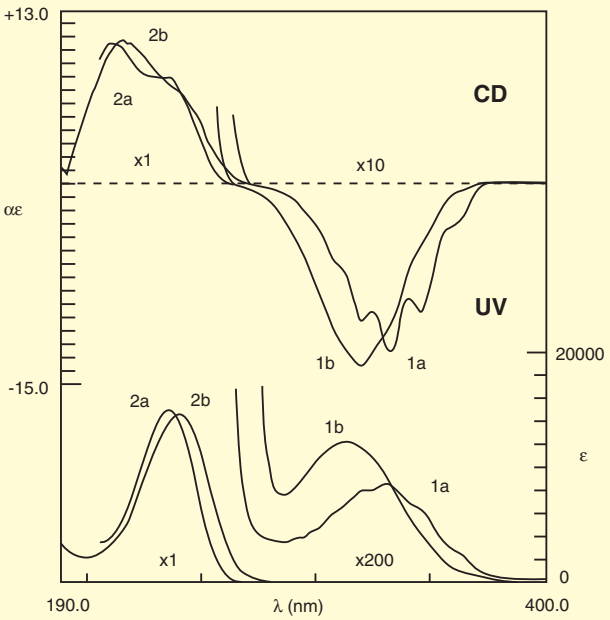


The octant projection chart of the A-ring.

The Cotton effect: R-band(-) and K-band(+)

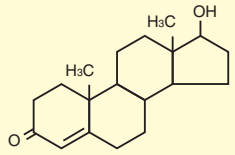
Figure 1 shows the CD/UV spectra of testosterone in dioxane, a nonpolar solvent and in ethanol, a polar solvent. The spectral bands observed are assigned to the chromophore of, α, β-unsaturated ketone in the molecule: with the increasing polarity of the solvent, the n-π* transition (R-band at 300 to 350 nm) shows a bathochromic effect together with the blue shift while the π-π* transition (K band at 230 to 240 nm) shows the red shift. Accordingly, dioxane is used in this series of measurements. On the other hand, the sign of CD spectra is determined by the chirality of the two double bonds conjugated each other.[2] In Δ^4-en-3-one-type steroids like testosterone, the A-ring takes a half-chair type conformation where its octant projection chart indicates the negative and positive Cotton effect for the R-band and K-band respectively,[2] as shown in the following figures, showing a good correspondence with the observed CD spectra in Figure 1.

Figure 1. The CD/UV and IR spectra of testosterone



Sample: Tokyo Kasei Kogyo GR
Measurement Apparatus
CD: J-720 Circular Dichroism Spectrophotometer
UV: Ubest V-550 Ultraviolet and Visible Light Spectrophotometer

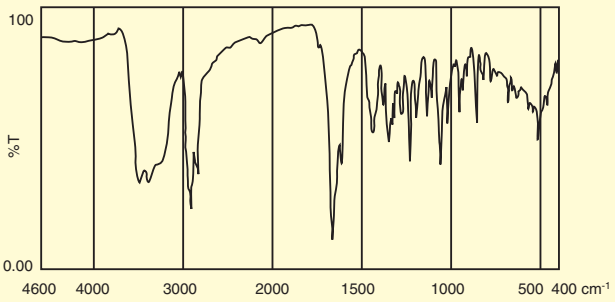
The structure of testosterone



17β-Hydroxyandrost-4-en-3-one
(Testosterone)

$C_{19}H_{28}O_2$=288.43

IR spectrum (KBr tablet method)



Measurement apparatus: FT/IR-300

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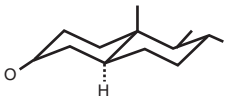
Tel: 0426-66-1321 FAX: 0426-65-6512 Web: http://www.jascoint.co.jp Mail: support1@jascoint.co.jp

CD spectra of pharmaceutical substances - Steroids (1)

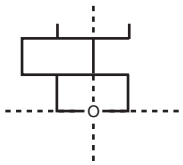
2. Stanolone

Stanolone (5α-dihydrotestosterone) is a metabolite derived from testosterone and is called agonist-type androgen because it essentially acts as a male hormone. This substance is not described in the Japanese Pharmacopoeia.

Figure 2 shows the CD/UV spectra of stanolone. Both of the spectral bands are assigned to the chromophore of saturated carbonyl group (n-π* transition of 280 to 300nm) and observed in the area of wavelength shorter than the R-band of α,β-unsaturated ketone. The positive sign observed in the CD spectrum can be explained by the application of the octant rule[2] about a saturated carbonyl group to both of the A and B rings in the structure of 5α-3-one-type steroids like stanolone, as shown in the following figures.



The half-chair type conformation of the A-ring



The octant projection chart of the A- and B-rings

The octant rule, (+) Cotton effect

3. Testosterone propionate

Testosterone propionate is used in intramuscular injections to treat hypogonadism caused by the lack of male hormones.[3] Testosterone propionate is a medicine, synthesized by the propionylation of the 17β-hydroxyl group in testosterone, for the purpose of increasing durability of action in the body.[3] Figure 3 shows its CD/UV spectra. Both of the spectra are similar to those of testosterone.

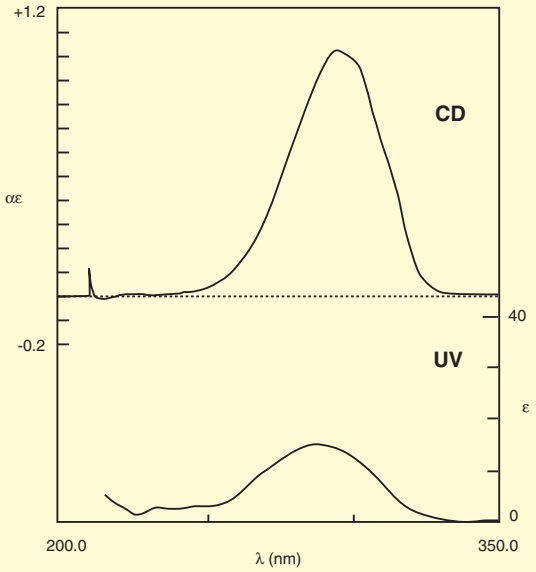
4. Methyltestosterone

Methyltestosterone is used orally to treat problems such as androgen deficiency.[3] It is the 17α-methylated derivative of testosterone and is known to be stronger than testosterone because it is not deactivated in the liver and therefore maintains action longer in a body.[3] Figure 4 shows its CD/UV spectra. Both of the spectra are similar to those of testosterone.

References

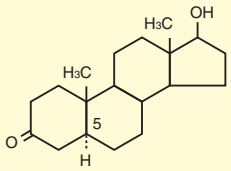
1) The Dictionary of Biochemistry, Tokyo Kagaku Dojin, 1st Edition, 1984.
2) Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry, Ed. by G. Snatzke, Heyden & Son Ltd., 1967
3) The Manual of Japanese Pharmacopoeia, 12th Edition, Hirokawa Shoten, 1991.

Figure 2. The CD/UV and IR spectra of stanolone



Sample: Tokyo kasei Kogyo GR A-0462
Dioxane Solution. 5.0 mg/10 ml(1.7 mM)
10 mm Cell
Measurement Apparatus
CD: J-720 Circular Dichroism Spectrophotometer
UV: Ubest V-550 Ultraviolet and Visible Light Spectrophotometer

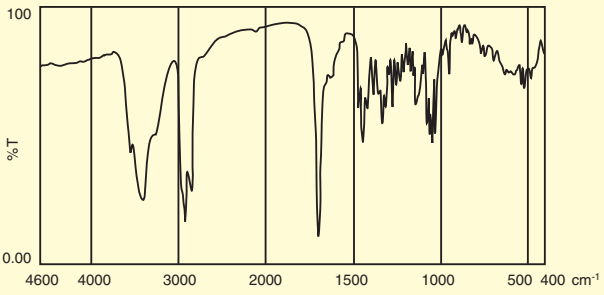
The structure of stanolone



5αAndrostan-17β-ol-3-one
(Stanolone=5a-Dihydrotestosterone)

$C_{19}H_{30}O_2$=290.45

IR spectrum (KBr tablet method)



Measurement apparatus: FT/IR-300

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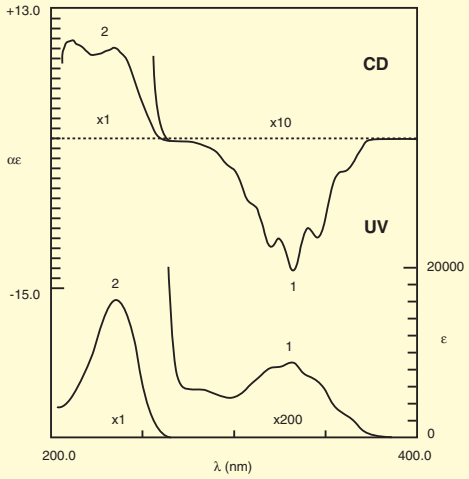
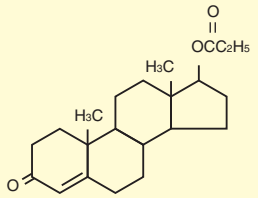
CD spectra of pharmaceutical substances - Steroids (1)

Figure 3. The CD/UV and IR spectra of testosterone propionate.



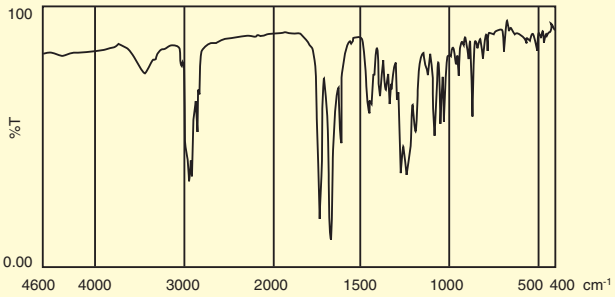
Sample: Tokyo Kasei Kogyo GR
Dioxane Solution.
1:5.0 mg/10 ml(1.5 mM), 10 mm Cell
CD:x10, UV:x200
2: 2.5 mg/10 ml(0.73 mM), 1 mm Cell
Measurement Apparatus
CD: J-720 Circular Dichroism Spectrophotometer
UV: Ubest V-550 Ultraviolet and Visible Light Spectrophotometer

The structure of testosterone propionate



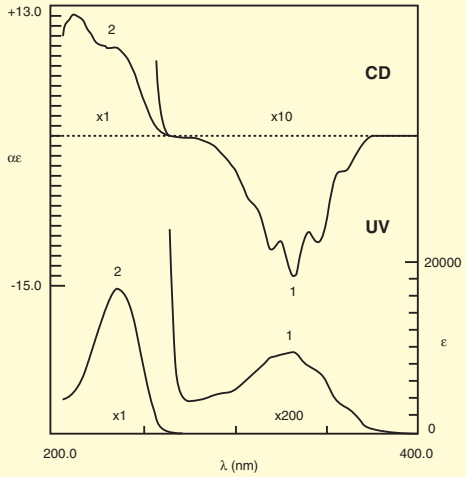
Testosterone propionate
$C_{22}H_{32}O_3$=44.49

IR spectrum (KBr tablet method)



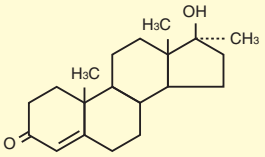
Measurement apparatus: FT/IR-300

Figure 4. The CD/UV and IR spectra of methyltestosterone



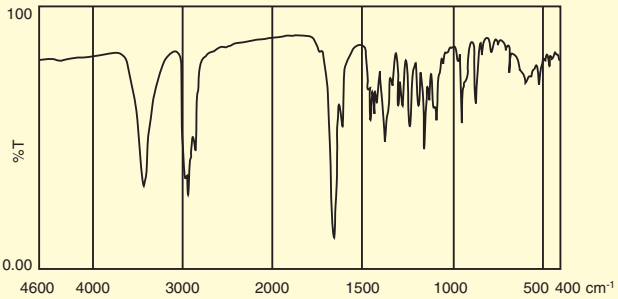
Sample: Wako reagent for biochemistry 136-09931
Dioxane Solution.
1 : 4.9 mg/10 ml(1.6 mM), 10 mm Cell
CD : x10, UV:x200
2 : 2.45 mg/10 ml(0.80 mM), 1 mm Cell
Measurement Apparatus
CD: J-720 Circular Dichroism Spectrophotometer
UV: Ubest V-550 Ultraviolet and Visible Light Spectrophotometer

The structure of methyltestosterone



17β-Hydroxy-17α-Methylandrost-4en-3-one(Methyltestosterone)
$C_{20}H_{30}O_2$=302.46

IR spectrum (KBr tablet method)



Measurement apparatus: FT/IR-300

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