RESOLUTION OF RACEMIC 3-METHOXY-14α-HYDROXY-D-HOMO--1,3,5(10)-ESTRATRIEN-17a-ONE*

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For the resolution of racemic 3-methoxy-14α-hydroxy-D-homo-1,3,5(10)-estratrien-17a-one the authors applied the method of reduction with chiral hydrides, chromatography on optically active material (acetyl cellulose), and reduction with microorganisms (*Saccharomyces cerevisiae* and *Rhizopus nigricans*). The first method gave poor yield, the second was suitable mainly for analytical purposes (separation of micro quantities), and the third gave satisfactory preparative and optical yields.

In their studies on total syntheses of steroids Torgov and coworkers[1] described the total synthesis of racemic 3-methoxy-14α-hydroxy-D-homo-1,3,5(10)-estradien-17a-one (*I*) in which they expected physiological activity. In view of the fact that their product was racemate and that the activity may be expected rather or exclusively in the enantiomer corresponding to the natural estrone, we tried to resolve the racemate *I*. As *I* is a substance of neutral character it was impossible to make use of the classical procedure based on the preparation of salts with an optically active component. Therefore we used for the resolution *1*) asymmetric reduction with chiral hydrides, *2*) chromatography on optically active material (acetyl cellulose), and *3*) resolution with biological material, according to the principle devised by Wettstein[2,3]. The first method gives 17a-alcohols which have to be reoxidised to the starting material, the second can afford the required enantiomers directly, the third gives both alternatives.

Reduction of the 17a-oxo group with lithium aluminum hydride in the presence of (−)-quinine or (−)-menthol according to Červinka[4] leads to a mixture of epimeric 17a-alcohols (*II* and *III*). In the case of the use of (−)-quinine the optical yield of the products obtained was low (3·4 and 5·7%) while in the case of (−)-menthol the rotation was zero within experimental error.

When racemate *I* was chromatographed on a thin layer of acetyl cellulose[5–7] in the conventional arrangement an imperfect separation took place. Only when the wedge-strip technique was applied on the thin-layer a complete separation of the

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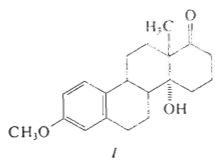
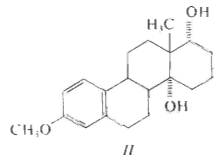
enantiomers was achieved. The differences in R_F values of the enantiomers were small but sufficient for analytical purposes. Chromatography on a column of acetyl cellulose of the usual dimensions gave low optical yields (4 and 7% when ratio of the mixture to the sorbent was 1 : 200).

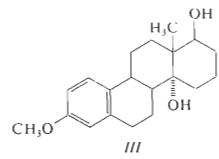
In our attempts at the resolution of racemate *I* with biological material a series of microorganisms and plant tissues was tested for their ability to reduce the 17a-oxo group (Table I). For preparative experiments we chose baker's yeast (*Saccharomyces cerevisiae*) and the mould *Rhizopus nigricans*. Both microorganisms have been used for specific reductions rather often[8,9]. The less polar product of reduction of racemic ketone *I* with yeast had its R_F value, melting point, analyses and IR spectra identical with those of racemic alcohol *II* obtained by reduction of racemic *I* with lithium aluminum hydride. The more polar product had the same data identical with racemic alcohol *III* also obtained by $LiAlH_4$ reduction. From alcohols *II* and *III* the substances obtained by reduction with yeast differed merely by their optical rotation. Oxidation of both optically active alcohols with chromium trioxide gave enantiomeric ketones *Ia* and *Ib*. Alcohol *II* gave ketone *Ib* of $[\alpha]_D^{20}$ +132° and $\Delta\varepsilon_{295} = +2{\cdot}64$, while alcohol *III* afforded ketone *Ia* of $[\alpha]_D^{20}$ −125° and $\Delta\varepsilon_{295} = -2{\cdot}76$. The circular dichroism curves of ketones *Ia* and *Ib* were mutual mirror images. According to thin-layer chromatography on acetyl cellulose both enantiomers were pure. Enantiomer *Ib* had a higher and the other one a lower mobility. Hence,



I



II



III

Formulae

In formulae *I-III* the number without further indication means a racemate with index *a* means the enantiomer corresponding to the natural estrone, represented by the formula, and with index *b* the other enantiomer.

the resolution of the racemic ketone *I* to optically pure antipodes using the microbiological method was successful and the yield was relatively good (32%).

The study of models and the applications of the octant rule led to the assignment of the absolute configuration to both enantiomers: the dextrorotatory ketone is *ent*-3-methoxy-14α-hydroxy-D-homo-1,3,5(10)-estratrien-17a-one (*Ib*), formed by oxidation from *ent*-3-methoxy-14α,17aα(S)-dihydroxy-D-homo-1,3,5(10)-estratriene (*IIb*), while the levorotatory ketone *Ia* obtained on oxidation of 3-methoxy-14α,17aβ(*S*)-dihydroxy-D-homo-1,3,5(10)-estratriene (*IIIa*) corresponds in its absolute configuration to the natural estrone. From this it follows that the alcoholdehydrogenase from *Saccharomyces cerevisiae* is specific with respect to the reaction centre at C_{17a}. Both alcohols formed possess *S*-configuration, in agreement with the finding of Prelog[10] for some other dehydrogenases.

In view of the fact that on reduction of the racemic compound *I* with yeast under various conditions (excess of yeast, time of transformation) a part of the starting product always remained unreacted (and was optically inactive) we also used for the

TABLE I

Transformation with Biological Material

Spots of the products on silica gel thin-layer plates: R_F *I* 0·56, (starting material), *II* 0·42, *III* 0·30.

No	Material	*I*	*II*	*III*	Other spots
	Moulds and fungi				
1	*Rhizopus nigricans* EHRENBERG	+	−	+	−
2	*Curvularia lunata* (WAKKER) BOEDIJN	−	+	+	+
3	*Absidia coerulea* BAINIER	−	−	+	++
4	*Aspergillus niger* VAN TIEGHEM	+	−	+	−
5	*Penicilium notatum* WESTLING	++	−	++	+
6	*Saccharomyces cerevisiae* MEYEN ex HANSEN	+	++	++	−
	Parts of higher plants				
7	*Lactuca sativa* L., var *capitata* (leaves)	+	−	+	+
8	*Capsicum annuum* L. (fruit core)	+	−	++	−
9	*Pisum sativum* L. (seedlings)	+	−	++	++
10	*Cucumis sativus* L. (fruits)	+	−	−	−
11	*Faba vulgaris* MOENCH. (young leaves)	+	−	++	−
12	*Faba vulgaris* MOENCH. (young roots)	+	−	++	−
13	*Solanum tuberosum* L. (tubers)	++	−	−	−

+ Weak spot, ++ strong spot, − absence of spot.

reduction of racemic *I* *Rhizopus nigricans* which according to preliminary experiments transformed ketone *I* in higher yields and practically only to one epimer at 17a, *i.e.* *IIIa*. As the unreacted starting product displayed a positive optical rotation the conclusion may be made that the alcoholdehydrogenase from *R. nigricans* attacks only the enantiomer with the configuration of the natural series, giving the product in a fairly good yield (41%). Therefore this strain may be considered more suitable for the preparation of the enantiomer with the configuration corresponding to the natural series from racemate *I* than *S. cerevisiae*. From the fact that *R. nigricans* attacked only the enantiomer with the configuration of the natural series it may be considered as substrate specific.

Finally, it should be pointed out that the sign of optical rotation of the products obtained on reduction of *I* with chiral hydrides ((−)-quinine) was the opposite from that of the products obtained on reduction with microorganisms, *i.e.* the mentioned hydride gave alcohols with prevailing *R*-configuration.

Hence, it would be interesting to study the relationship between the chemical structure of chiral hydrides and the signs and the yields of the products of asymmetric reduction of various ketones and see whether the results could be somehow correlated with the results of reductions with microorganisms.

EXPERIMENTAL

The melting points were measured on a Kofler block and were not corrected. Samples for analyses were dried at 20°C/0·2 Torr for 15 hours. Optical rotation was measured in chloroform on a polarimeter Opton (Germany). The infrared spectra were measured on a UR-10 (Zeiss, Jena) spectrophotometer, in chloroform. The circular dichroism curves were recorded with a Jouan-Roussel Dichrographe II. Thin layer chromatography on silica gel (according to Stahl, Merck) was carried out in chloroform–methanol (98 : 2) (S_1), that on acetyl cellulose (MN 300 AC/c. 10%; Macherey, Nagel and Co., Düren) in methanol–water (1 : 1) (S_2). Preparative chromatography on plates (20 × 20 cm) with a layer of silica gel was carried out in chloroform, using double development. Acetyl cellulose for preparative column chromatography MN 2100 AC/c. 10% was from Macherey, Nagel and CO., Düren. The moulds and fungi were from the Collection of Cultures of Moulds and Fungi at the Department of Botany, Charles University, Prague.

Transformation of Racemic 3-Methoxy-14α-hydroxy-D-homo-1,3,5(10)-estratrien-17a-one (*I*) with Microorganisms and Plant Tissues

a) *Test with submersed cultures* (Table I, 1—5): 10 ml of a medium composed of 5 g corn-steep, 11 g glucose, 0·4 g KH_2PO_4, and 0·4 g $MgSO_4$ per 1 litre of water were poured into half-litre flasks and sterilised. Sterile spore suspensions (3—4 ml) of the tested microorganisms were added and cultivated on a shaker at 20—24°C for 48 hours. A solution of racemate *I* (1 mg) in methanol (0·2 ml) was then added to the grown mycelium and shaking was continued at the same temperature for another 24 hours. The suspension was then filtered and the filtrate extracted with 30 ml of chloroform. The extract was dried over sodium sulfate and evaporated. The residues dissolved in 0·1 ml of chloroform were applied on a silica gel thin-layer plate and chromatographed in system S_1.

b) *Test with plant tissues* (Table I, 6—10): 3 g of plant tissue were cut into small pieces and suspended in 10 ml of the above nutrient medium, one mg of racemate *I* was added and the mixture shaken at room temperature for 24 hours. The suspension was filtered and the filtrate worked up as above.

c) *The test with baker's yeast* was carried out with commercial baker's yeast similarly as under *b*); the amount of yeast (100, 250 and 500 mg per 10 ml of water or nutrient solution) and the time of shaking (3, 6, 10, 16, 20, 24, 36 and 48 hours) were varied in order to find optimum conditions for the reduction of *I*. The suspension was extracted with chloroform without previous filtration. The best yields of reduction with yeast were achieved when using 250 mg of yeast per 1 mg of substance, distilled water as medium and 16—20 hours incubation time.

Reduction of Ketone *I* with Lithium Aluminum Hydride

Lithium aluminum hydride (50 mg) was added in portions to a stirred solution of ketone *I* (100 mg) in tetrahydrofuran (5 ml). The stirring was continued for another 15 minutes and excess hydride was decomposed with a saturated solution of sodium sulfate. The suspension was dried over sodium sulfate, filtered, and the residue on the filter washed with chloroform. After evaporation of the solvent the residue was submitted to preparative chromatography on two thin-layer plates. The zones of both racemic alcohols *II* and *III* formed were scratched off, eluted with chloroform and evaporated. The residue of the eluate of *II* (44 mg) was crystallised from methanol, m.p. 205—207°C (literature[1] gives 202—203°C from ethyl acetate). IR spectrum: ν(OH) free: 3610, 3624 cm^{-1}; ν(OH) bonded: 3533 cm^{-1}. The residue of eluate *III* (42 mg) was crystallised from ethyl acetate, m.p. 198—199·5°C (literature[1] gives m.p. 196—197°C from ethyl acetate). In the IR spectrum the band from the free hydroxyl group was found only: ν(OH) free: 3620, 3630 cm^{-1}.

Reduction of Racemic Ketone *I* with *Saccharomyces cerevisiae*

A solution of ketone *I* (50 mg) in methanol (22 ml) was added in portions (5 ml each) at 15 minutes' intervals to a suspension of yeast (12·5 g) in distilled water (750 ml). The suspension was shaken for 20 hours, extracted with two 500 ml portions of chloroform and the extract was washed with 5% sodium hydrogen carbonate solution, dried over sodium sulfate, filtered, and evaporated. The residue was separated on preparative thin-layer plates (2 plates). After elution of the zone, corresponding to the products, and further conventional working up, two alcohols were obtained faster moving *IIb* and slower moving *IIIa*.

Alcohol *IIb*, m.p. 198—200°C from methanol, $[\alpha]_D^{20}$ +30·8° (*c* 1·7). The IR spectrum was identical with that of alcohol *II*. The same is true of the R_F values and colour changes during the detection with sulfuric acid and heating.

Alcohol *IIIa*, m.p. 203—206°C, $[\alpha]_D^{20}$ −33·2° (*c* 0·8). The IR spectrum was identical with that of alcohol *III*. The same is true of the R_F values and reaction with sulfuric acid.

Oxidation of Alcohols *IIb* and *IIIa* with Chromium Trioxide

To a solution of alcohol *IIb* (25 mg) in acetone (1·5 ml) Jones' reagent was added dropwise under cooling until the yellow coloration presisted. The excess reagent was decomposed with methanol. After working up of the reaction mixture the product was purified by preparative chromatography on silica gel thin-layer (one plate). The eluate of the zone of the oxidation product was crystallised

from methanol, and ketone *Ib* was thus obtained, m.p. 189—190°C, $[\alpha]_D^{20}$ +132° (*c* 1·0); CD: $\Delta\varepsilon$ +2·64 (295 nm).

Using the above procedure alcohol *IIIa* (25 mg) was oxidised to ketone *Ia* which after crystallisation from methanol had m.p. 189—190°C, $[\alpha]_D^{20}$ −125° (*c* 1·0); CD: $\Delta\varepsilon$ −2·76 (295 nm).

Separation of Racemic Ketone *I* on Acetyl Cellulose

a) *On thin-layer*: On a plate (30 × 5 cm; wedge shaped) with acetyl cellulose, 25 μg of ketone *I* were chromatographed in S_2. Detection was carried out by spraying with conc. sulfuric acid and mild heating in an oven until yellow zones appeared. The zones also fluoresced under the UV lamp. The zone of higher R_F value belongs to ketone *Ib*, the zone of lower R_F value to the enantiomer *Ia*.

a) *On a column.* Acetyl cellulose (20 g) was packed in dry state into a column of 2 cm diameter, and height of sorbent 60 cm. The racemic ketone *I* (100 mg) was chromatographed on it in system S_2 and fractions of 20 ml volume were collected. In the second fraction after the beginning of elution of ketone *Ib* CD measurements gave the value $\Delta\varepsilon$ +0·11 (295 nm), corresponding to an optical yield of about 4·1%. In the subsequent two fractions ketone *Ia* was eluted the maximum value of which $\Delta\varepsilon$ −0·19 (295 nm) corresponded to optical yield 6·9%.

Transformation of Racemate *I* with *Rhizopus nigricans*

Six one-litre flasks containing 200 ml of the sterile nutrient medium each (see above) were inoculated with spore suspension of *R. nigricancs* and cultivated on a reciprocal shaker at 20°C for 24 hours. A solution of ketone *I* (10 mg in 1 ml of methanol per flask) was then added to the flasks and the shaking continued at the same temperature for another 20 hours. The suspension was filtered and the combined filtrates extracted with chloroform. The extract was washed with a small amount of 5% sodium hydrogen carbonate, dried over sodium sulfate, filtered and evaporated. The residue was separated on preparative thin-layer plates (2 plates). The zone of the alcohol *IIIa* formed was scraped off and eluted. The eluate (25 mg) was crystallised from ethyl acetate, m.p. 203—205°C, $[\alpha]_D^{20}$ −32° (*c* 1·5). The IR spectrum was identical with that of alcohol *III* or *IIIa* obtained by reduction of ketone *I* with yeast. The unreacted starting compound gave crystals of $[\alpha]_D^{20}$ +71·6° (*c* 1·0) from methanol proving that ketone *Ia* was not reduced with *R. nigricans* quantitatively.

Reduction of Racemic Ketone *I* with Chiral Hydrides

To a solution of lithium aluminum hydride (67 mg) in ether (30 ml) (−)-quinine (550 mg) was added under stirring followed by dropwise addition of a solution of racemate *I* (150 mg) in tetrahydrofuran (3 ml). After five hours' boiling under reflux the reaction mixture was worked up in the usual manner and the crude product separated on preparative silica gel thin-layer plates. Both alcohols were thus obtained, *II* (53 mg) and *III* (42 mg). After their oxidation with Jones' reagent their rotations and CD spectra were measured. Ketone *I* from alcohol *II* had $[\alpha]_D^{20}$ −9·7° (*c* 1·5), CD: $\Delta\varepsilon$ −0·16 (295 nm), corresponding to a yield of *Ia* about 5·7%. Ketone *I* from alcohol *III* had $[\alpha]_D^{20}$ +5·5° (*c* 1·3), CD: $\Delta\varepsilon$ +0·1 (295 nm), corresponding to a yield of ketone *Ib* about 3·9%.

In the same manner ketone *I* was also reduced with chiral complex hydride prepared from (−)-menthol, but the products isolated had no optical activity.

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