We believe that the present work also illuminates the structural characteristics required to endow 1,3-dipole reactivity and suggests interesting extensions. Further work is in progress.

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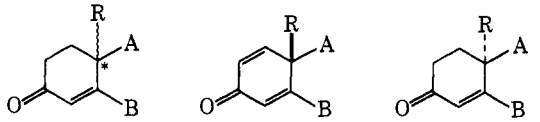
The Substrate Selectivity of the Steroid Dehydrogenase of *Arthrobacter simplex*. Its Use for the Resolution and Determination of Absolute and Relative Configuration in Total Synthesis

Sir:

The total synthesis of polycyclic hydroaromatic systems, including steroids and other terpenoids, involves the more or less stereoselective generation of new asymmetric centers whose stereochemistry relative to the remaining centers is often unknown. Available methodology for ascertaining the configuration of the new center is limited, X-ray crystallography often constituting the only reliable procedure.

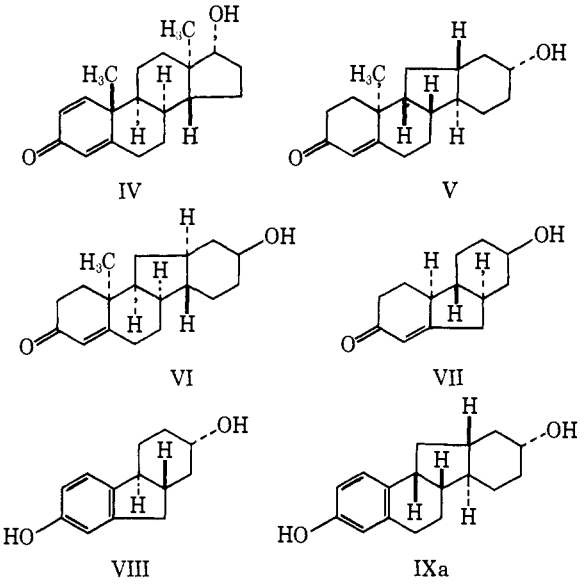
We wish to report a novel and rapid procedure, which resolves racemic mixtures, indicating in the process the absolute configuration of the two enantiomers at the newly generated center, and by ORD or CD measurements provides the absolute configuration of the remaining centers, the net result being the complete stereochemical description of both enantiomers. The systems amenable to such treatment are α,β-unsaturated ketones of the general structure I where R may be H or CH_3 and A and B together represent the remaining elements of a polycyclic system totaling two, three, or four rings, the latter being either five or



I II III

six membered. Such systems may be the result of a Birch reduction, of a Robinson or Stork annellation, etc.

The critical reagent is the steroid dehydrogenase of *Arthrobacter simplex*, which in the form of an acetone-dried cell powder represents a stable material. We have found that in all cases examined the enzyme attacks preferentially that antipode of a racemate which possesses the center marked with an asterisk in the configuration corresponding to that of C-10 in "natural" steroids[1] to form II,[2] leaving unchanged III. The following is typical of this enzymatic resolution. To 30 ml of a suspension of *A. simplex* cells[3,4] (40 mg/ml) in 0.01 *M* pH 7.0 phosphate buffer prepared with the aid of a Waring blender was added with stirring a solution of 15 mg of *rac*-9β,10α-testosterone and 2 mg of 2-methyl-1,4-naphthoquinone in 1.5 ml of ethanol. After stirring for 24 hr at 25° the mixture was extracted with ethyl acetate–acetone (1:1, 30 ml) and centrifuged, and the organic layer separated. After two more extractions with ethyl acetate the organic layers were washed with water, dried, and evaporated *in vacuo*. The residue (20.2 mg) after tlc on silica gel (ethyl acetate–chloroform 1:9, developed three times) yielded IV [(4.4 mg), mp 175–176°; $[\alpha]_D$ +16.5°;[5] M^+ 286] and "natural" *l*-9β,10α-testosterone, mp 150–152°; $[\alpha]_D$ −138°; reported[6] mp 154–156°; $[\alpha]^{diox}_D$ −141°. From the above result and the fact that *l*-nortestosterone



remains unchanged during incubation with a cell-free preparation of *A. simplex*,[7] while its "natural" enantiomer is converted to estradiol, it was conjectured that chirality at C-10 was decisive for recognition of the substrate by the enzyme. This was verified with all substrates examined and will be illustrated in connection with the total synthesis of the C-nor-D-homosteroids *rac*-V and *rac*-VI described recently.[8]

Starting material was the tricyclic ketone *rac*-VII whose configuration at C-10 was uncertain.[9] Enzymatic dehydrogenation of *rac*-VII for 5 days gave after tlc separation the phenol VIII and recovered VII. The phenolic methyl ether of VIII had CD, $[\theta]_{225}$ +7100, $[\theta]_{280}$ +960, B-norestradiol, $[\theta]_{227}$ +7100, $[\theta]_{284}$ +2000. Jones oxidation of the methyl ether afforded the ketone,[8] CD, $[\theta]_{220}$ +7800, $[\theta]_{288}$ +5800, the increase in θ_{288} indicating the strong positive Cotton effect due to the new keto group. The recovered VII had $[\theta]_{320}$ −180. "Natural" B-nor-Δ^4-cholestenone possesses a positive Cotton effect near 320 nm.[10] The above CD data establish the absolute configuration of VIII and of the corresponding chiral centers in VII.

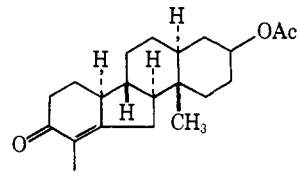
(1) In most cases this is the *R* configuration.
(2) All structural formulae designate the enantiomer whose absolute configuration is shown.
(3) W. Umbreit, R. H. Burris, and J. F. Stauffer, "Manometric Techniques," Burgess, Minneapolis, Minn., 1957, p 164.
(4) S. C. Pan, J. Semar, B. Junta, and P. A. Principe, *Biotechnol. Bioeng.*, **11**, 1183 (1969).
(5) All $[\alpha]_D$ and $[\theta]$ values in methanol unless otherwise indicated. ORD and CD measurements were performed on a Cary 60 instrument.
(6) A. M. Krubiner, G. Saucy, and E. P. Oliveto, *J. Org. Chem.*, **33**, 3548 (1968).
(7) L. Penasse and M. Peyre, *Steroids*, **12**, 525 (1968).
(8) M. J. Green, N. A. Abraham, E. B. Fleischer, J. Case, and J. Fried, *Chem. Commun.*, 234 (1970).
(9) J. Fried and N. A. Abraham, *Tetrahedron Lett.*, 3505 (1965).
(10) C. Djerassi, R. Riniker, and B. Riniker, *J. Amer. Chem. Soc.*, **78**, 6377 (1956).

Making now the well-founded assumption that the enzyme prefers the antipode of VII possessing 10*R* configuration leads to the prediction of *anti-trans* stereochemistry for VII. This is in conformity with the X-ray crystal structure for *rac*-V, which latter incorporates these three centers unchanged as C-8, C-13, and C-14 by virtue of the synthetic route.

A further test for the preference of the enzyme for the 10*R* configuration involved the assignment of relative configuration at C-10 to the two isomeric pairs, *rac*-V and *rac*-VI derived from *rac*-VII. Both *rac*-V and *rac*-VI were shown from nmr data to possess rings B and C in *cis* fusion after conversion to IX and its enantiomer IXa, respectively, by the action of the *A. simplex* enzyme (4 hr and 5 days, respectively) followed by Dryden aromatization.[8] *rac*-V and *rac*-VI differ, therefore, only in their relative stereochemistry at C-10. The phenol IXa derived from *rac*-VI had CD $[\theta]_{230}$ −1600, $[\theta]_{280}$ positive. In comparison, estradiol (9α) showed $[\theta]_{233}$ +5900, $[\theta]_{283}$ −390, and 9β-estradiol has a negative Cotton effect near 230 nm and a positive one near 280 nm.[11] IX derived from the enantiomer of V had Cotton effects opposite to those of IXa. The preference of the dehydrogenase for the antipodes of V and VI, which possess the same chirality at C-10 (but opposite chirality at all remaining centers), again points up the importance of this center for enzyme selectivity. The above data define the absolute configuration of IX and IXa, and of V and VI and their enantiomers with the exception of that at C-10.[12] Applying now our rule that the enantiomers possessing 10*R* chirality are the preferred substrates for dehydrogenation, IXa must be derived from *rac*-VI, while its enantiomer IX has as its precursor *rac*-V. This defines the complete stereochemistry of V, VI, and their enantiomers, and is in full accord with the structure of *rac*-V as determined by X-ray crystallography.[8]

It is significant that the examples presented in this paper include tricyclic and tetracyclic systems, as well as systems containing five-membered rings. It should be pointed out that only when R = CH_3 (structure I) does the enzyme show complete specificity. When R = H it shows selectivity,[13] and when chirality at C-10 is eliminated as by introduction of a 9,10 double bond both enantiomers are attacked at equal rates.[8]

It was of interest to demonstrate that the 10*R* selectivity rule was valid for bicyclic systems as well. Dehydrogenation of *rac*-Δ^4-9-methyloctalin-3,8-dione followed by reisolation of the substrate showed the latter to have $[\alpha]_D$ −33°, and the 8-ol derived from it by $NaBH_4$ reduction[14] to possess θ_{318} +730. (9*R*)-Δ^4-9-Methyloctalin-3,8-dione has $[\alpha]^{EtOH}_D$ −130°,[15] and (8*S*,9*S*)-Δ^4-9-methyloctalin-3-on-8-ol shows a negative Cotton effect near 320 nm.[16] The 9*S* enantiomer (corresponding to 10*R* chirality in previous examples) is thus again preferentially dehydrogenated.[17]



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(11) P. Crabbé, "Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry," Holden-Day, San Francisco, Calif., 1965, p 293.

(12) The CD curves for recovered V and VI were also determined but their sign is of questionable value in determining absolute configuration, because of the influence of subtle conformational factors on the sign of the Cotton effects of steroidal α,β-unsaturated ketones (*cf.* G. Snatzke, *Tetrahedron*, **21**, 421 (1965)). V and VI were therefore oxidized to the corresponding diketones and the Cotton effect for the saturated keto group was determined as the difference between the CD curves for V and VI and their respective diketones. These Cotton effects should provide a safe indication of the absolute configuration of all centers other than C-10. The values for $\Delta\theta_{285}$ were −4900 and +400, respectively, which confirms the conclusions derived from the CD data for IX and IXa.

(13) This results in incomplete resolution and lower than maximal values for the ORD and CD peaks. This in no way alters the validity of the proposed procedure.

(14) C. H. Heathcock, R. A. Badger, and J. W. Patterson, Jr., *J. Amer. Chem. Soc.*, **89**, 4133 (1967).

(15) V. Prelog and W. Acklin, *Helv. Chim. Acta*, **39**, 748 (1956).

(16) C. Djerassi, J. Osiecki, and W. Herz, *J. Org. Chem.*, **22**, 1361 (1957).

(17) It was gratifying to find that *rac*-**1** (J. P. Kutney, J. Cable, W. A. F. Gladstone, H. W. Hanssen, E. J. Torupka, and W. D. C. Warnock, *J. Amer. Chem. Soc.*, **90**, 5332 (1968)) on dehydrogenation for 5 days left **1**, CD, θ_{248} +6300, θ_{318} −2200. Authentic **1** (W. F. Johns and I. Laos, *J. Org. Chem.*, **30**, 123 (1965)) had θ_{248} +45,700, θ_{318} −16,600.

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Stable Carbonium Ions. CIII.[1] Ring Contraction and Transannular Bond Formation in Medium-Ring Cycloalkyl Cations

Sir:

Solvolyses of medium-ring (eight- to eleven-membered) cycloalkyl derivatives occur at enhanced rates over the common-ring (five- to seven-membered) and large-ring (12+-membered) cycloalkyl derivatives. These results have been interpreted as due to relief of steric strain.[2] From labeling experiments, Prelog has shown that facile transannular hydride shifts occur after ionization but before solvent capture in the solvolyses.[2] Acetolysis of cyclodecyl tosylate yields mainly *cis*- and *trans*-cyclodecenes, in the ratio of 1:5. It was observed that decomposition of solid cyclodecyl tosylate also gave small amounts of deca-

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(2) (a) V. Prelog, *J. Chem. Soc.*, 420 (1950); (b) R. Heck and V. Prelog, *Helv. Chim. Acta*, **38**, 1541 (1955); (c) V. Prelog and J. G. Traynham in "Molecular Rearrangements," Part 1, P. deMayo, Ed., Wiley-Interscience, New York, N. Y., 1963, pp 593–615; (d) V. Prelog, *Rec. Chem. Progr.* **18**, 247 (1957); as in "Nonclassical Ions," P. D. Bartlett, Ed., W. A. Benjamin, New York, N. Y., 1965, pp 197–210; (e) V. Prelog, W. Küng, and T. Tomljenovic, *Helv. Chim. Acta*, **45**, 1352 (1962); (f) H. C. Brown and C. Ham, *J. Amer. Chem. Soc.*, 78, 2735 (1956).