300°. All are yellow. The pentamer softens at about 90°. The properties of these substances will be described in greater detail in a full publication.

These oligomers were separated by dry column chromatography[14] on an inactive grade of silica gel[15] using carbon disulfide as the developer, the R_f decreasing with increasing polymerization. They were isolated from the sectioned chromatography column by Soxhlet extraction, the dimer, trimer, and tetramer with carbon disulfide, the pentamer with toluene.

The total yield of purified material was about 18%, of which approximately 10% was the di-, 20% the ter-, 30% the quater-, and 40% the quinqueferrocenophane.

This work demonstrates that the first four [1^n]ferrocenophanes can be made by a polygemination reaction, and it seems reasonable to suppose that other and larger polymers might be made in this way.

Acknowledgments. We are grateful to the National Institutes of Health and the National Science Foundation for support.

(14) B. Loev and M. M. Goodman, *Chem. Ind.* (London), 2026 (1967).
(15) Activity II silica gel[14] (Baker No. 3405) plus 84 g of water/lb.

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Stereochemistry of Estrogen Biosynthesis

Sir:

The loss of the angular methyl group at C-10 and of one hydrogen each from C-1 and C-2 represents the structural changes involved in the biosynthesis of estradiol from neutral C-19 precursors. Studies of this transformation have so far failed to reveal the exact nature and sequence of these events but have established that hydroxylation of the methyl group precedes its expulsion and that the hydrogen lost from C-1 is stereospecifically β.[1] In an effort to throw further light on this most significant androgen to estrogen biotransformation we have investigated the stereochemistry of the hydrogen loss from C-2 and report our results in this communication.

The required aromatization substrates stereoselectively labeled with tritium in the 2β and 2α positions were prepared by the following sequences. Treatment of 3β-tosyloxy-5α,6α-epoxyandrostan-17-one (I) with Li_2CO_3 in dimethylacetamide gave 5α,6α-epoxyandrost-2-en-17-one (II). Reduction of II with lithium aluminum hydride afforded androst-2-ene-5α,17β-diol (III) which on oxidation with *m*-chloroperbenzoic acid gave 2α,3α-epoxyandrostane-5α,17β-diol (IV). Reduction of IV with [^{3}H]-$LiAlH_4$ yielded [2β-^{3}H]3α,5α,17β-triol V; acetylation and subsequent dehydration with thionyl chloride in pyridine provided [2β-^{3}H]androst-4-ene-3α,17β-diol diacetate (VI). Mild alkaline hydrolysis of VI followed by oxidation with the Jones reagent[2] at 0° gave one of the required precursors, [2β-^{3}H]androst-4-ene-3,17-dione (VII). The same compound with the isotope in the epimeric 2α orientation was prepared by a route in which the olefin II also served as the starting material. Hydroboration of II with tritiated diborane[3] and subsequent oxidation gave a mixture of products from which, after careful separation, pure [2α-^{3}H]3α-hydroxy-5α,6α-epoxyandrostan-17-one (VIII) was obtained. The conversion of VIII to the other substrate, [2α-^{3}H]androst-4-en-3,17-dione (IX), proceeded analogously to that described above for the 2β-labeled material VII. The well-documented *trans* mechanism of lithium aluminum hydride reduction of epoxides[4] and the *cis* nature of hydroboration[5] allowed confidence in the stereochemical assignment of the isotope in each instance.[6] It should be emphasized that the above syntheses were so designed that the introduction of the 3-ketone and hence labilization of the α-methylene at C-2 was the last step in each sequence. This limited the exposure of the introduced tritium to enolization and served to preserve the integrity of the label as confirmed by the constancy of the specific activity prior to and after the oxidation in both instances.[7] Substrates VII and IX were shown to be homogeneous by reverse isotope dilution analysis and were devoid of radioactivity following alkali treatment. To permit ready determination of tritium loss upon incubation both VII and IX were mixed with an appropriate amount of [^{14}C]androsten-4-ene-3,17-dione to obtain substrates with a specific tritium to carbon-14 ratio.

The incubations were carried out with human placental microsomes prepared according to Ryan.[8] The incubation mixture contained, in addition to the microsomes and 100–150 μg of steroid substrate, a NADPH-generating system consisting of 10 μM NADPH, 120 μM glucose 6-phosphate, and 9.6 units of glucose 6-phosphate dehydrogenase. The incubations were carried out at pH 7.2 at 37° for 1 hr and terminated by the addition of acetone. Products and recovered starting material were isolated by dilution with carrier, separation by quantitative thin layer chromatography, and recrystallization to constant isotope ratio.

The results of the incubations are listed in Table I. The estrone obtained from the substrate VII with the isotope in the 2β orientation exhibited an 83% loss of

Table I. Stereochemistry of C-2 Hydrogen Loss in Placental Aromatization

	Substrate	
	2β-^{3}H (VII)	2α-^{3}H (IX)
Substrate $^3H/^{14}C$[a]	11.1	5.8
Estrone $^3H/^{14}C$	1.9	5.4
% ^{3}H lost	83	7
Recovered substrate $^3H/^{14}C$	12.0	7.3

[a] Expressed as ratio of counts per minute.

(1) P. Talalay, *Ann. Rev. Biochem.*, 34, 347 (1965), and references cited therein.
(2) A. Bowers, T. G. Halsall, E. R. H. Jones, and A. J. Lemin, *J. Chem. Soc.*, 2548 (1953).
(3) Prepared *in situ* from sodium borotritiide and boron trifluoride etherate.
(4) L. W. Trevoy and W. G. Brown, *J. Am. Chem. Soc.*, 71, 1675 (1949); M. Mousseron, R. Jacquier, M. Mousseron-Canet, and R. Zagdoun, *Bull. Soc. Chim. France*, 1042 (1952); G. M. Helmkamp and B. F. Rickborn, *J. Org. Chem.* 22, 479 (1957).
(5) H. C. Brown, and G. Zweifel, *J. Am. Chem. Soc.*, 81, 247 (1959).
(6) The stereochemical assignments were confirmed by *Bacillus sphaericus* dehydrogenations, the results of which will be presented in the full paper.
(7) Oxidations with the Jones reagent in other similar situations also proceeded without loss of isotope by enolization: J. Fishman, *J. Am. Chem. Soc.*, 87, 3455 (1965); J. Ramseyer and H. Hirschmann, *J. Org. Chem.* 32, 1850 (1967).
(8) K. J. Ryan, *J. Biol. Chem.*, 234, 268 (1959).

tritium. Conversely, the 2α-tritiated substrate IX suffered only a 7% loss. These figures establish that the hydrogen loss at C-2 in the aromatization process is highly stereoselective and is β, and hence the overall C-1,2 dehydrogenation is *cis*. The starting material recovered from both incubations exhibited an increase in tritium content, indicating the presence of primary and secondary isotope effects. More importantly, however, the retention of tritium confirms that the hydrogen removal from C-2 is not a reversible process under the conditions employed.

Present concepts[9] of the mechanism of enzymatic aromatization imply that the hydrogen loss from C-2 proceeds *via* enolization as one step in the biotransformation sequence. The removal of the axial 2β hydrogen may be considered in accord with the enolization mechanism since such is the preferred stereochemistry of enolization. However, the irreversibility of the hydrogen loss suggests that the enolization, if such it is, takes place concurrently or subsequently to the expulsion of the C-19 methyl group since then the driving force of the aromatization would prevent reversal of the enol formation. Alternatively, enolization need not be involved in the hydrogen loss at C-2 which may instead be linked to that at C-1 by a dehydrogenation sequence. The demonstrated *cis* nature of this process is in agreement with the stereochemistry of dehydrogenation at other sites of the steroid molecule.[10] It is hoped that further clarification of this and other aspects of the aromatization mechanism will result from work now in progress.

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(9) J. D. Townsley and H. J. Brodie, *Biochemistry*, 7, 33 (1968).
(10) A. M. Paliokas and G. J. Schropfer, *J. Biol. Chem.*, **243**, 453 (1968).

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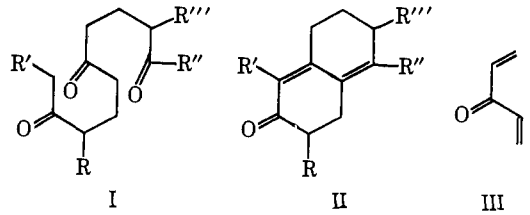
β-Chloroethyl Vinyl Ketone, a Useful Reagent for the Facile Construction of Fused Ring Systems

Sir:

Multiple intramolecular aldol condensations, where the consecutive carbonyl groups are arrayed in a 1,5 pattern, provide an interesting potential for the facile construction of fused six-membered rings. If this concept, generalized as I → II, could be reduced to practice, it could find extensive application in the synthesis of natural products. We now report (i) an effective method for generating a 2,6,10-triketo system and (ii) its utility in the production of a tricyclic system in high yield.



I II III

In principle, a system such as I could be assembled *via* successive Michael additions of appropriate carbonyl systems to divinyl ketone (III, DVK). In practice, mono Michael addition has never been successfully conducted with DVK.[1] Fragmentary evidence obtained in our laboratory suggests that the reason lies in the greater electrophilicity of an alkyl vinyl ketone relative to DVK itself. Hence bisalkylation of DVK is, at minimum, competitive with monoalkylation—a situation which adversely affects the smooth production of an unsymmetrical 2,6,10-trione such as I.

We now report that β-chloroethyl vinyl ketone[2] (IV, CVK) is an effective synthetic equivalent of DVK (III). The compound,[3] bp 25° (0.08 mm), is prepared in 37% yield by a two-step sequence involving acylation of ethylene with β-chloropropionyl chloride followed by controlled monodehydrohalogenation of the crude 1,5-dichloro-3-pentanone so produced.[4] Its nmr (neat) spectrum [τ 3.58–4.33 (3 H), nine-line multiplet; 6.28 (2 H), t, J = Hz; 7.00 (2 H), t, J = 7 Hz] is in accord with the assigned structure.

We have studied the reaction of the sodium salt of V with CVK under a variety of conditions. Preliminary evidence strongly suggests that Michael addition occurs at a substantially faster rate than either alkylation by the primary chloride or dehydrohalogenation (formation of DVK). Thus, in an ether–water mixture, the initial product obtained is compound VI.[5] Its structure follows from its ir [$\lambda_{max}^{CHCl_3}$ 5.69, 5.80, and 5.85 μ], nmr [τ 6.48 (2 H), t, J = 7 Hz (CH_2Cl)], and mass [m/e 230 (P), 232 (P + 2)] spectra. In monoglyme the Michael adduct suffers dehydrohalogenation resulting in the formation of VII[3,6,7] (bp 100° (0.03 mm); mp *ca.* 17°; λ_{max}^{EtOH} 211 mμ (ε 9000)) in crude yield[8] of 85%. Its ir spectrum (CCl_4) exhibits maxima at 5.65, 5.80, 5.92, and 6.19 μ and its nmr (CCl_4) contains a 3H nine-line multiplet from τ 3.67 to 4.38 (vinylic protons).

Reaction of VII with *t*-butyl acetoacetate in *t*-butyl alcohol containing catalytic amounts of potassium *t*-butoxide afforded a gummy product which, upon treatment with TsOH–HOAc at 78° for 3 hr, gave a

(1) Bisalkylation of DVK was reported in its base-catalyzed reaction with 2-methyldihydroresorcinol: I. Nazarov and S. I. Zavyalov, *Zh. Obsch. Khim.*, **23**, 1703 (1953). Bisalkylation where the second step is intramolecular has been employed by Wynberg and coworkers as an interesting route to spiro systems: H. A. P. DeJongh and H. Wynberg, *Rec. Trav. Chim.*, **82**, 202 (1963).
(2) CVK is mentioned in the literature as an impurity in various preparations of DVK and 1,5-dichloro-3-pentanone: J. R. Miller and E. M. Wilkinson, British Patent 789,128 (1958), *Chem. Abstr.*, **52**, P9669*b* (1958); G. Baddeley, H. T. Taylor, and W. Pickles, *J. Chem. Soc.*, 124 (1953).
(3) Combustion analyses within 0.3% of theory were obtained for this compound.
(4) The method of acylation was developed by N. Jones and H. T. Taylor, *J. Chem. Soc.*, 1345 (1961). The modification came at the dehydrohalogenation step. We employed a 1.1:1 ratio of sodium carbonate:dichloropentanone and milder distillation conditions than the British workers.
(5) This compound was obtained as an oil which could not be purified for analysis by distillation. The presence of VII as an impurity was suggested by its nmr spectrum.
(6) That VII did not arise from alkylation by the primary halide is suggested from the studies of Rosenthal,[7] who observed predominantly *ortho* alkylation in the reaction of V with alkyl halides.
(7) D. Rosenthal and K. H. Davis, Jr., *J. Chem. Soc.*, 1973 (1966).
(8) Examination of the nmr spectrum of the crude material suggests the presence of small amounts of compound VI. Compound VII can be purified by vacuum distillation. This reduces its yield to 60%.