Table I. Comparative Distribution of Methylene Insertion Products

Substrate	Product	% calcd.	% obsd. From I	% obsd. From II	% obsd. From CH_2N_2[a]
Pentane	2-MeC_5	33.3	34		34
	3-MeC_5	16.7	17		17
	C_6	50.0	49		49
3-Methylpentane	3,3-Me_2C_5	7.1		7	7
	2,3-Me_2C_5	28.6		30	31
	3-MeC_6	42.9		43	44
	3-EtC_5	21.4		20	18
2,4-Dimethylpentane	2,2,4-Me_3C_5	12.5		10	10
	2,4-Me_2C_6	75.0		77	78
	2,3,4-Me_3C_5	12.5		13	12
2,4-Dimethylhexane	2,2,4-Me_3C_6	5.6	6		5
	[2,4,4-Me_3C_6 / 2,3,5-Me_3C_6][b]	16.7	17		16
	2,4-Me_2C_7	16.7	18		19
	2-Me-4-EtC_6	16.7	16		16
	3,5-Me_2C_7	33.3	34		35
	2,3,4-Me_3C_6	11.1	9		10
2,2,4-Trimethylpentane	2,2,4,4-Me_4C_5	5.6	4	5	4
	2,2,4-Me_3C_6	33.3	37	36	35
	2,4,4-Me_3C_6	50.0	49	49	51
	2,2,3,4-Me_4C_5	11.1	10	10	10
2,3,4-Trimethylpentane	2,2,3,4-Me_4C_6	11.1	10	11	10
	2,4-Me_2-3-EtC_5	16.7	14	16	16
	2,3,4-Me_3C_6	66.7	70	67	69
	2,3,3,4-Me_4C_5	5.6	6	6	5

[a] CH_2N_2 data for pentane taken from ref. 2; other CH_2N_2 data taken from ref. 5 or present study. [b] Incomplete resolution.

Apart from the theoretical significance of these new precursors, they constitute a convenient, shelf-stable source of active methylene. The hydrocarbon precursors are thus particularly suitable for small-scale synthesis[16] and C^{14} labeling[18] *via* methylene insertion and should facilitate application of these techniques to substrates which undergo dark reactions with the functional methylene precursors.

Acknowledgment. We are grateful to Mr. L. M. Taylor for experimental assistance and to Dr. F. D. Mango for helpful comments.

(18) M. A. Muhs, E. L. Bastin, and B. E. Gordon in "Advances in Tracer Methodology," Vol. 2, S. Rothchild, Ed., Plenum Press, New York, N. Y., 1965, p. 39.

(19) Mesa College, Grand Junction, Colo.

(20) Shell Development Company, Emeryville, Calif.

Davis B. Richardson, Larry R. Durrett, James M. Martin, Jr., William E. Putnam,[19] Stephen C. Slaymaker, Isaac Dvoretzky[20]

Shell Oil Company, Houston Research Laboratory
Deer Park, Texas 77536

Received April 15, 1965

An Efficient Synthesis of Estrone and 19-Norsteroids from Cholesterol

Sir:

The increasing medical importance of 19-norsteroids, particularly as prostagens[1] for ovulation control, has generated intensive research to find more economical routes, either by direct partial or total synthesis or indirectly *via* estrone, to this class of compounds.[2]

(1) G. Pincus and A. P. Merrill in "Control of Ovulation," C. A. Villee, Ed., Pergamon Press, New York, N. Y., 1961, p. 37.

In our previous communication[3] we described the feasibility of the cleavage of the sterol side chains by microbial means, thus providing an alternate source of starting materials for the preparation of steroid hormones. It was shown that 19-hydroxycholest-4-en-3-one and 19-hydroxysitost-4-en-3-one could be converted into estrone by microorganisms. As an extension of this finding, we herein report an improved and highly efficient route for the preparation of estrone and 6,19-oxidoandrost-4-ene-3,17-dione (I), a key intermediate for the synthesis of 19-norsteroids[4] from cholesterol.

Previous studies have shown that in general most bacteria contain enzyme systems which are capable of (1) cleaving acetoxyl functions at C-3[5]; (2) oxidation of hydroxyl groups at C-3 to ketones;[6] (3) isomerization of the double bond[7] ($\Delta^{5,6}$ to $\Delta^{4,5}$); (4) introduction of a 1,2-double bond[8,9]; (5) cleavage of the cholesterol side chain in a series of reactions which eventually lead to a 17-ketone function.[3,10] When these isolated facts are brought into focus, it appears to us that an ideal substrate for the microbial conversion to estrone would be 3β-acetoxy-19-hydroxycholest-5-ene[4] (II) since this compound could be conveniently prepared from cholesterol acetate in three chemical steps. Also, by taking advantage of all the enzymes produced by the microorganism, a shorter synthesis to estrone could be realized.

In a typical experiment, 1.2 g. of II was incubated with CSD-10[11] for 96 hr. in Difco nutrient broth; 527 mg. of estrone (72%), m.p. 257–260°, was obtained (identity with an authentic sample established by mixture melting point and infrared spectrum).

We have previously shown that I could be converted into 6,19-oxido-9α-hydroxyandrost-4-ene-3,17-dione (III) by the organism *Nocardia restrictus* (ATCC 14887).[12] However, the presence of the 6,19-oxido function in the molecule apparently blocked the introduction of the 1,2-double bond by the microorganism, resulting in the accumulation of the product, III. On the basis of this observation, it occurred to us initially that 6,19-oxidocholest-4-en-3-one (IV) should be a substrate for microbial conversion, but the major product of this microbial transformation could be either I or III. When 900 mg. of IV was incubated with CSD-10 for 70 hr., 375 mg. (57%) of I was obtained, m.p. 182–185° (identical with an authentic specimen with respect to mixture melting point and infrared spectrum), along with 150 mg. of a mixture consisting mainly of III, m.p. 263–266°, and a small amount of a dihydro derivative of III.

In an attempt to devise a more efficient synthesis of I, by similar lines of reasoning, we predicted that 3β-acetoxy-5-chloro-6,19-oxidocholestane[4] (V) would be an ideal substrate for microbial transformation into I; V

(2) T. B. Windholz and M. Windholz, *Angew. Chem.*, 76, 249 (1964).

(3) C. J. Sih and K. C. Wang, *J. Am. Chem. Soc.*, **87**, 1387 (1965).

(4) J. Kalvoda, K. Heusler, H. Ueberwasser, G. Anner, and A. Wettstein, *Helv. Chim. Acta*, **46**, 1361 (1963).

(5) C. J. Sih, J. Laval, and A. M. Rahim, *J. Biol. Chem.*, **238**, 626 (1963).

(6) P. Talalay and P. J. Marcus, *ibid.*, **218**, 675 (1956).

(7) F. S. Kawahara and P. Talalay, *ibid.*, **235**, PC 1 (1960).

(8) H. R. Levy and P. Talalay, *ibid.*, **234**, 2014 (1959).

(9) C. J. Sih and R. E. Bennett, *Biochim. Biophys. Acta*, **56**, 584 (1962).

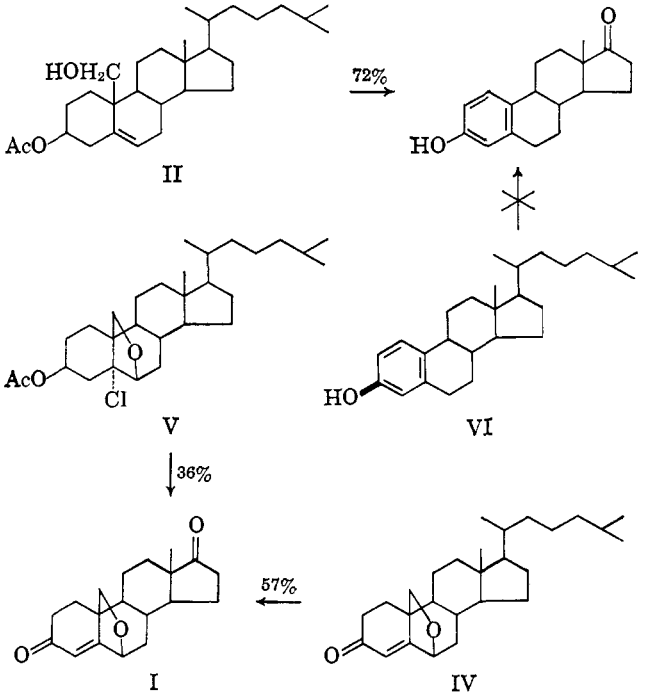
(10) J. M. Whitmarsh, 435th meeting of the Biochemical Society, Dec. 1963.

(11) CSD-10 is a microorganism isolated from soil utilizing cholesterol as a sole carbon source.

(12) C. J. Sih, S. S. Lee, Y. Y. Tsong, and K. C. Wang, *J. Am. Chem. Soc.*, **87**, 1385 (1965).

could be prepared from cholesterol acetate in two steps. The only uncertainty at this stage would be the effect of the chlorine atom at position 5 on the microorganism. However, the presumed intermediate, β-chloro ketone, should eliminate HCl easily even in the fermentation medium. When 1.1 g. of V was exposed to CSD-10 for 80 hr., 250 mg. (36%) of I, m.p. 179–182°, was obtained.

Surprisingly, 3-hydroxycholesta-1,3,5(10)-triene (VI) was not metabolized by CSD-10.[13]



It is our contention that the major pathway of cholesterol degradation by this microorganism must first involve the removal of the side chain to yield a C-19 steroid which is then metabolized *via* the 9,10-seco pathway of Dodson and Muir.[14] By blocking the introduction of the 1,2-double bond through the formation of a 6,19-oxido bridge or by interfering with the 9α-hydroxylation process through the introduction of a hydroxyl function at C-19,[15] the desired intermediate, I, and estrone accumulated. Table I shows that androst-4-ene-3,17-dione is an effective inducer and substrate for both the 9α-hydroxylase and 1-dehydrogenase. On the other hand, cholest-4-en-3-one apparently is an extremely poor inducer of both of these enzymes and could not act as a substrate for the 9α-hydroxylase. But it is a substrate for the 1-dehydrogenase. This is consistent with the fact that we have been unable to detect the presence of 9α-hydroxylated products in the C-27 series whereas a small quantity of VI was detected when 19-hydroxycholest-4-en-3-one was used as the substrate.

The results herein presented describe a four-step synthesis of estrone and a three-step synthesis of I from cholesterol acetate. To our knowledge, this probably constitutes the most economical processes to date for the preparation of estrone and 19-norsteroids.[16]

(13) It is interesting to note that Turfitt reported [*Biochem. J.*, **42**, 376 (1948)] that many *Proactinomyces* strains, after prolonged culture upon estradiol as sole carbon source, showed a diminution in their capacity for oxidizing cholesterol.

(14) R. M. Dodson and R. D. Muir, *J. Am. Chem. Soc.*, **83**, 4627 (1961).

(15) 19-Hydroxyandrost-4-ene-3,17-dione is considerably less efficient as an inducer and substrate for the 9α-hydroxylase than androst-4-ene-3,17-dione.

Table I. Induction and Specificity of Ring Cleavage Enzymes

Inducer	Substrate	% relative activity A[a]	B[b]
Androstenedione	Androstenedione	100	100
Androstenedione	Cholestenone	<1	<1
Cholestenone	Cholestenone	<1	<1
Cholestenone	Androstenedione	9[c]	<1

[a] A, 9α-hydroxylase activity; B, 1-dehydrogenase activity. [b] These data were abstracted from ref. 9. [c] This activity is probably due to the conversion of cholestenone into androstenedione, which is a good inducer of 9α-hydroxylase.

(16) This investigation was supported in part by research grants from the Wisconsin Alumni Research Foundation and the National Institutes of Health (AM-4874 and AM-6110)

Charles J. Sih, S. S. Lee
Y. Y. Tsong, K. C. Wang, F. N. Chang
School of Pharmacy, University of Wisconsin
Madison 6, Wisconsin
Received April 1, 1965

Electron and Energy Transfer in Irradiated Xenon–Hexane Liquid Solutions

Sir:

We have measured the dimer and hexene yields in Co^{60} γ-irradiated xenon–hexane liquid solutions at −78° and compared them with those for pure hexane.[1] Both dimers and hexenes are formed in yields corresponding to essentially complete electron energy transfer even at 0.04 mole fraction of hexane in xenon. Furthermore, the distribution of the six dimers and five hexenes is similar for all mole fractions studied. Thus we can conclude that the precursors of the observed products must be substantially the same whether they arise from energy transfer or direct radiolysis. We postulate that electron transfer from hexane to ionized xenon is the predominant mechanism of energy transfer (which process is exothermic, 1.7 e.v. in the gas phase[2]), and that the product precursors are hexane ions, although this can hardly be said to have been established beyond question. We believe our results demonstrate in a simple way a very efficient energy transfer in a novel liquid system. Previously, Bouldin and Gordy[3] demonstrated energy transfer in krypton matrices at 4.2°K. containing 10^{-4} and 10^{-1} mole fraction of methane by e.s.r. observation of trapped hydrogen atoms, and Borkowski and Ausloos[4] studied the effects of adding noble gases on the radiolysis of gaseous isobutane. Electron transfer is much more easily studied in gas mixtures by mass spectrometry, and the rate constants so determined are among the fastest known in all of reaction kinetics.[5,6] Electron transfer can occur rapidly over distances of several atomic diameters even when tunneling is required.[6]

(1) (a) L. Kevan and W. F. Libby, *J. Chem. Phys.*, **39**, 1288 (1963); (b) H. Widmer and T. Gaumann, *Helv. Chim. Acta*, **46**, 944 (1963); (c) *ibid.*, **46**, 2780 (1963).

(2) F. H. Field and J. L. Franklin, "Electron Impact Phenomena," Academic Press Inc., New York, N. Y., 1957.

(3) W. V. Bouldin and W. Gordy, *Phys. Rev.*, **135**, A806 (1964).

(4) R. P. Borkowski and P. J. Ausloos, *J. Chem. Phys.*, **38**, 36 (1963).

(5) F. W. Lampe, J. L. Franklin, and F. H. Field, "Progress in Reaction Kinetics," Vol. 1, Pergamon Press, London, 1961, p. 69.

(6) R. A. Marcus, *Ann. Rev. Phys. Chem.*, **15**, 155 (1964).