produced in the heterogeneous reaction of Engel and Skell.[6,13] Present evidence from a consideration of the energetic atom case, the condensed-phase surface reaction, and the photolytic case would suggest that a proper combination of spin state and kinetic energy will allow a carbon atom to insert in a C–H bond.

(13) Other things being equal, any spin restriction in the product-determining step would apply to all three methods of producing cyclopropylcarbinyl carbene.

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Microbiological Synthesis of 16-Keto Steroids from Steroidal Sapogenins

Sir:

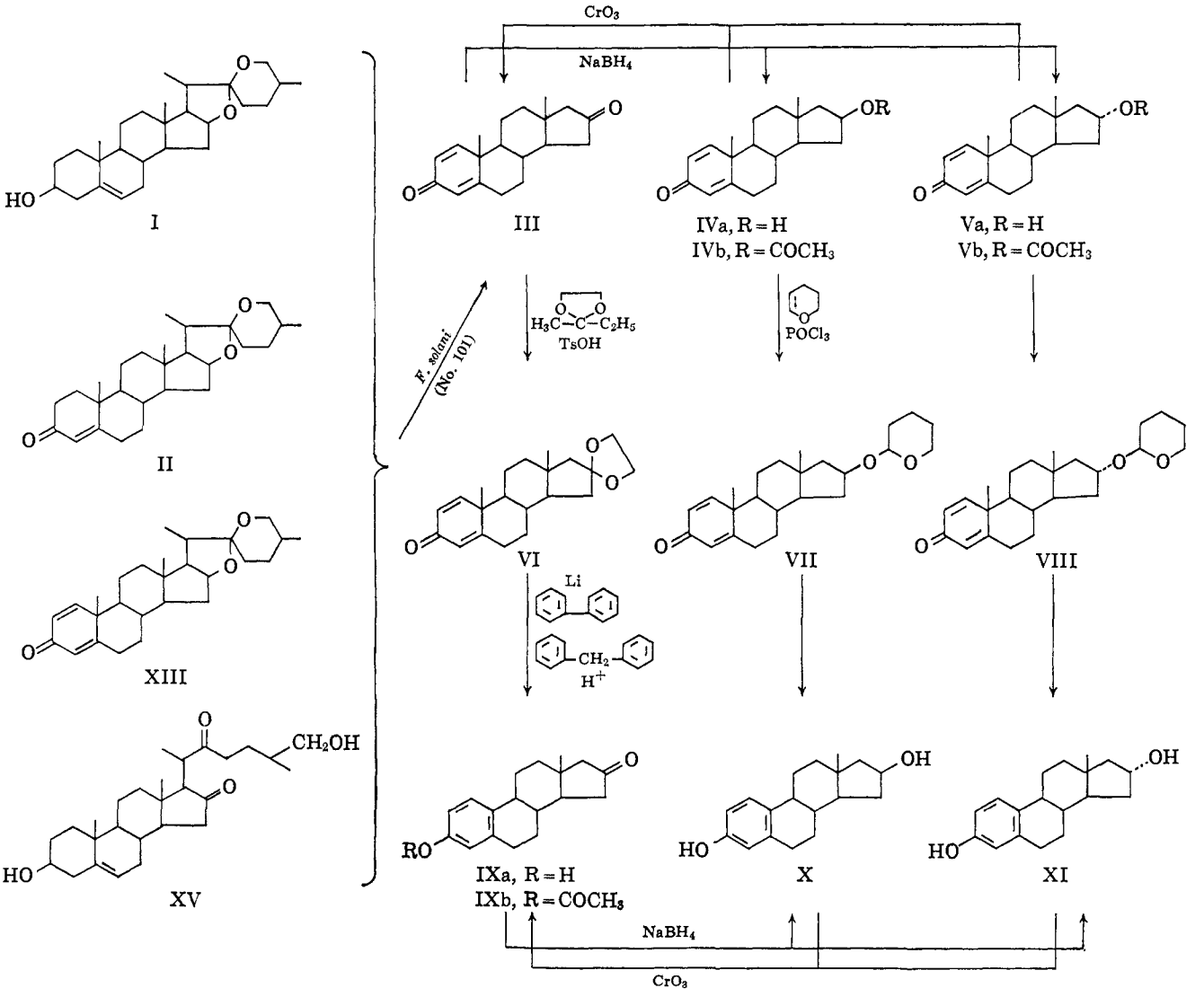
It has long been known that the steroidal sapogenins are good sources for chemical preparation of steroidal hormones. As far as the microbiological utilization of sapogenins is concerned, however, only a few investigations have been reported.

Mininger and his co-workers[1] attempted to transform commonly occurring sapogenins with microorganisms and concluded that steroidal sapogenins are not readily attacked. The first successful microbiological hydroxylation (7β and 11α) of diosgenin (I) was reported by Hayakawa and Satoh.[2] Dehydrogenation of I to form 25D-spirost-4-en-3-one (II, diosgenone) and 25D-spirosta-1,4-dien-3-one (XIII, 1-dehydrodiosgenone) was recently announced by Iizuka and Iwafuji.[3]

We wish to report a new microbiological transformation of sapogenins dealing with the degradation of the spiroketal ring in the initially formed Δ^1-dehydrogenation products (Scheme I).

Incubation of II with *Fusarium solani* (No. 101)[4] afforded three crystalline compounds, III, IVa, and Va, in yields of about 65, 5, and 5%, respectively. These compounds were also obtained from I, but the yields were very low. Analytical samples showed the following constants:[5] III ($C_{19}H_{24}O_2$), mp 140–141°, $[\alpha]^{24}D$ −204.2°, $\lambda_{max}^{95\%EtOH}$ 245 mμ (ϵ 15,400), ν_{max}^{Nujol}

Scheme I



(1) R. F. Mininger, M. E. Wall, R. G. Dworschack, and R. W. Jackson, *Arch. Biochem. Biophys.*, 60, 427 (1956).

(2) S. Hayakawa and Y. Satoh, *J. Org. Chem.*, 28, 1742 (1963).

(3) H. Iizuka and Iwafuji, Meeting of the Agricultural Chemical Society, Japan, April 1965.

(4) The medium consisting of 3.5% glucose, 2% peptone, and 0.3% corn steep liquor is suitable for growth.

(5) Elemental analyses of the described compounds gave satisfactory values. Unless otherwise noted, optical rotations were determined in chloroform containing 1% ethanol.

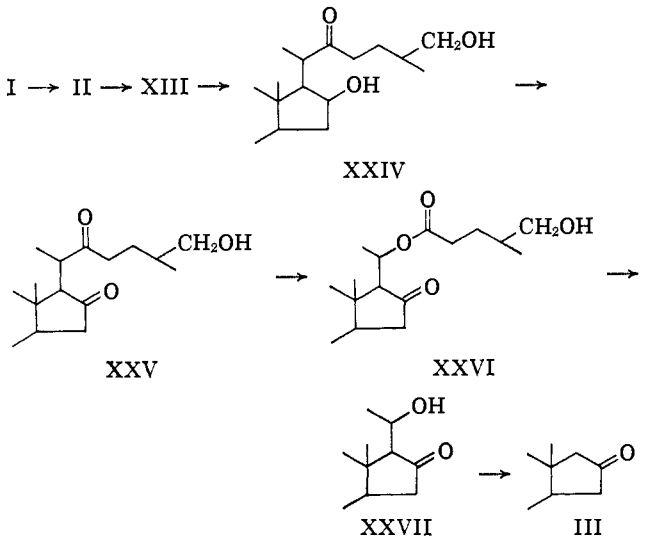
1730, 1658, 1622, and 1603 cm^{-1}; IVa ($C_{19}H_{26}O_2$), mp 201–202°, $[\alpha]^{22.5}D$ −15.2°, $\lambda_{max}^{95\%EtOH}$ 245.5 mμ (ε 16,000), ν_{max}^{Nujol} 3390, 1654, 1617, and 1602 cm^{-1}; Va ($C_{19}H_{26}O_2$), mp 186–187°, $[\alpha]^{25}D$ −17.4°, $\lambda_{max}^{95\%EtOH}$ 245.5 mμ (ε 16,000), ν_{max}^{Nujol} 3396, 1695, 1619, and 1602 cm^{-1}.

Compounds IVa and Va were converted into their corresponding monoacetates: IVb [$C_{21}H_{28}O_3$, mp 100–101°, $[\alpha]^{25}D$ −22.4°, $\lambda_{max}^{95\%EtOH}$ 245.5 mμ (ε 16,100), ν_{max}^{Nujol} 1733, 1664, 1624, and 1604 cm^{-1}] and Vb (syrup, $\nu_{max}^{CCl_4}$ 1737, 1666, 1632, and 1607 cm^{-1}). Oxidation of IVa or Va with CrO_3 gave a compound identical in all respects with III. Furthermore, $NaBH_4$ reduction of III afforded IVa and Va. These results indicate that the hydroxyl groups in IVa and Va are located on the same carbon atom in the D ring. The ORD curve of III in $CHCl_3$ showed a negative cotton effect ($[\phi]^{22.5}_{323}$ −13,520°, $[\phi]^{22.5}_{291}$ +7890°; a −214), indicating the five-membered ring keto group in III is in position 16.[6] The nmr spectrum of III in $CDCl_3$ showed the angular methyl signals (τ 9.03 for 18-methyl and τ 8.74 for 19-methyl), but did not show other signals corresponding to the 21- and 27-methyl groups presented in the substrate (I or II). From these facts, III was assigned the structure androsta-1,4-diene-3,16-dione and the alcohol (IVa or Va) the epimeric 16(α- or β-)hydroxyandrosta-1,4-dien-3-one.

The following series of reactions were used to establish their structures. Transformation of androst-4-ene-3,16-dione[7] (XII) with *F. solani* (No. 101) or other microorganisms[8] capable of introducing the 1,2-double bond gave III efficiently. Treatment of III with 2-methyl-2-ethyl-1,3-dioxolane[9] afforded an ethylene ketal (VI), which was converted by Dryden's method[10] into the aromatic compound IXa [$C_{18}H_{22}O_2$, mp 248–249°, $\lambda_{max}^{95\%EtOH}$ 281.5 mμ (ε 2190) and 287.5 mμ (ε 1920), ν_{max}^{Nujol} 3380, 1720, 1608, 1582, and 1499 cm^{-1}]. Physical properties of IXa and its acetate IXb [$C_{20}H_{24}O_3$, mp 132–133°, $[\alpha]^{22}D$ −91.7° (ethanol), ν_{max}^{Nujol} 1760, 1734, 1615, 1582, and 1496 cm^{-1}] agreed with those of 3-hydroxy-1,3,5(10)-estratien-16-one and its acetate,[11,12] respectively. Reductive aromatization[10] of pyranyl ethers VII and VIII, prepared from IVa and Va, respectively, afforded the corresponding epimers X [$C_{18}H_{24}O_2$, mp 230–231°, $[\alpha]^{22}D$ +72° (ethanol), ν_{max}^{Nujol} 3528, 3238, 1609, and 1506 cm^{-1}] and XI [$C_{18}H_{24}O_2$, mp 228–229°, $[\alpha]^{22}D$ +80° (ethanol), ν_{max}^{Nujol} 3372, 3125, 1613, and 1503 cm^{-1}]. Identity of X with 3,16β-dihydroxy-1,3,5(10)-estratriene[13] was established by mixture melting point, infrared spectra, and chromatographic behavior. Accordingly, IVa must be 16β-hydroxyandrosta-1,4-dien-3-one. Oxidation of X or XI with CrO_3 afforded IXa. In addition, X and XI were obtained from IXa with $NaBH_4$. These results leave little doubt that Va and XI are 16α-hydroxyandrosta-1,4-dien-3-one and 3,16α-dihydroxy-1,3,5(10)-estratriene,[14] respectively.

Finally, in order to clarify the transformation pathway from I to III, the following compounds were incubated with *F. solani* (No. 101): 1-dehydrodiosgenone (XIII), tigogenone (XIV), kryptogenin (XV), 5α-dihydrokryptogenin diacetate (XVI), pseudodiosgenin (XVII), 16α-hydroxyprogesterone (XVIII), 16-ketoprogesterone (XIX), 3β-acetoxy-16α,20β-dihydroxypregn-5-ene (XX), 20β-acetoxypregn-4-ene-3,16-dione (XXI), 3β,16β,20α-trihydroxypregn-5-ene (XXII), and 3β-acetoxy-20α-hydroxypregn-5-en-16-one (XXIII). These experiments demonstrated the formation of III from XIII, XIV, XV, XVI, XXI, XXII, and XXIII. From other substrates (XVII, XVIII, XIX, and XX), III was not obtained. In parallel experiments using *Arthrobacter simplex* (ATCC 6946),[8] it was found that the formation of III from XIX and XXI was very easy, suggesting that the microbiological removal of the 17β side chain may proceed *via* a 16-keto-20-hydroxypregnane intermediate with the aide of aldolase. From these findings, it would appear that the pathway is that shown in Scheme II, although its complete elucidation demands further investigtion.

Scheme II



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(7) Prepared by Dr. W. Nagata of our research laboratory (unpublished).

(8) *Bacillus sphaericus* (ATCC 7055) and *Arthrobacter simplex* (ATCC 6946) were used. In pregnane substrates, reduction of the 20-keto group is also observed with *A. simplex*.

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(14) Physical constants of XI agreed with those of 3,16α-dihydroxy-1,3,5(10)-estratriene reported by M. N. Huffman and M. H. Lott, *J. Biol. Chem.*, **215**, 627 (1955).

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