[CONTRIBUTION FROM THE CHEMICAL LABORATORY, HARVARD UNIVERSITY]

An *i*-Steroid Hydrocarbon from Ergosterol[1]

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RECEIVED JUNE 20, 1952

Reinvestigation of a hydrocarbon resulting from dehydration of ergosterol with either phosphorus oxychloride or *p*-toluenesulfonyl chloride in pyridine and heretofore regarded as an ergostatetraene has shown that the substance is 3,5-cyclo-$\Delta^{6,8(14),22}$-ergostatriene (V), the first known *i*-steroid in the ergosterol series. Reaction of the hydrocarbon with hydrogen chloride and its acid-catalyzed hydration result in opening of the cyclopropane ring with formation of mixtures of ergosteryl chlorides or of ergosterol isomers; ergosterol-B_1 of reasonable purity has been isolated from the product of hydration. A hydrocarbon, first described by Windaus, resulting from acid-catalyzed dehydration of 3α- and 3β-hydroxy-$\Delta^{4,7,22}$-ergostatriene was investigated to see if it corresponded to a highly dextrorotatory, alumina-isomerized impurity accompanying the *i*-steroid V. The results indicate that the Windaus hydrocarbon is $\Delta^{4,6,8(14),22}$-ergostatetraene (XIV). Formulas attributed earlier to hydrocarbons resulting from dehydration of epialloergosterol (NaOAc–Ac_2O, see III) and of lumisterol (C_{10}-epimer of III) are consistent with present evidence. "Ergosterol-E" is shown to consist largely of a mixture of dihydroergosterols.

Three isomeric hydrocarbons, described as ergostatetraenes and derived from ergosterol by various dehydration procedures, have been reported from the Göttingen Laboratory.[2] The first of these was prepared during an investigation of various esters of ergosterol.[3] Attempted preparation of the phosphate ester by treatment of ergosterol with phosphorus oxychloride led to a hydrocarbon, ergostatetraene-A, $C_{26}H_{42}$, formed in yields as high as 80%. The tetraene was reported to isomerize when warmed with acetic anhydride or exposed to sunlight in the presence of eosin. The isomer, ergostatetraene-B, was later prepared[4] directly from ergosterol by treatment with *p*-toluenesulfonyl chloride in pyridine, without isolation of an intermediate tosylate; it has been observed as a minor product of acetylation of ergosterol.[5] The third isomer, ergostatetraene-C, was first obtained by attempted sodium and alcohol reduction of tetraene-C and originally described as a dihydro derivative[3] but later shown to be isomeric; identical material is obtained by refluxing tetraene-A in amyl alcohol without sodium.[6]

The three compounds have similar melting points but differ considerably in optical rotation (Table I). They have identical absorption maxima.

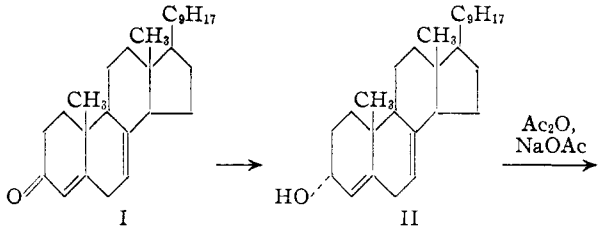
TABLE I

PROPERTIES OF THE HYDROCARBONS

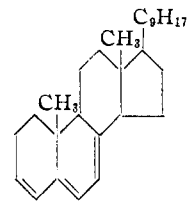
Ergostatetraene-	M.p., °C.	$\alpha^{20}D$ Chf	λ_{max}^{alc}, mμ(E)
A	97	+176°	260 (17,600)[a]
B	105	+ 93°	260 (26,800)[a]
C	98	+121°	260 (22,600)[a]

[a] Values observed in the present investigation at λ_{max}^{alc} 261 mμ.

Although the Windaus group proposed no structures, hydrocarbon A is listed in Elsevier[2] as $\Delta^{3,5,7,22}$-ergostatetraene (see formula III below), and such a structure could conceivably arise on dehydration of ergosterol. However, the observed absorption maximum is much too low for such a structure (calcd. 313 mμ). When lumisterol is dehydrated with either phosphorus oxychloride or *p*-toluenesulfonyl chloride, a tetraene is obtained[7] that has an absorption maximum at 314 mμ, a value compatible with a $\Delta^{3,5,7}$-triene system as in formula III. In the ergosterol series the Elsevier formula apparently belongs to an ergostatetraene prepared by dehydration of epialloergosterol[8] (II), one of two major products of Meerwein–Ponndorf reduction of ergosterone (I). The hydrocarbon



I Ergosterone — II Epialloergosterol



III $\Delta^{3,5,7,22}$-Ergostatetraene

resembles the Göttingen tetraene in melting point, but it is levorotatory (αD − 40.5°) and the principal ultraviolet absorption band is at 316 mμ (19,000), with subsidiary peaks at 301 and 331 mμ. The spectrum is almost identical with that of $\Delta^{3,5,7}$-cholestatriene, recently prepared by cleavage of 7-dehydrocholesteryl ethers with sodium alkyls.[9] The alternate $\Delta^{2,4,6}$-structure is also ruled out for the Göttingen hydrocarbons because substances having this triene system show a single peak in the ultraviolet at about 305 mμ (E about 15,000).[10]

(1) Based on the doctoral dissertation of William E. Rosen, May, 1952. The main results were presented in the symposium on steroids at the American Chemical Society Meeting, New York, September, 1951.

(2) See summary in Elsevier's "Encyclopedia of Organic Chemstry," Vol. 14, p. 22.

(3) O. Rygh, *Z. physiol. Chem.*, **185**, 99 (1929).

(4) W. Stoll, *ibid.*, **202**, 232 (1931).

(5) W. A. Stansbury, THIS JOURNAL, **65**, 1243 (1943).

(6) A. Guiteras, *Z. physiol. Chem.*, **215**, 196 (1933).

(7) I. M. Heilbron, F. S. Spring and P. A. Stewart, *J. Chem. Soc.*, 1221 (1935); I. M. Heilbron, G. L. Moffet and F. S. Spring, *ibid.*, 411 (1937).

(8) I. M. Heilbron, T. Kennedy, F. S. Spring and G. Swain, *ibid.*, 869 (1938).

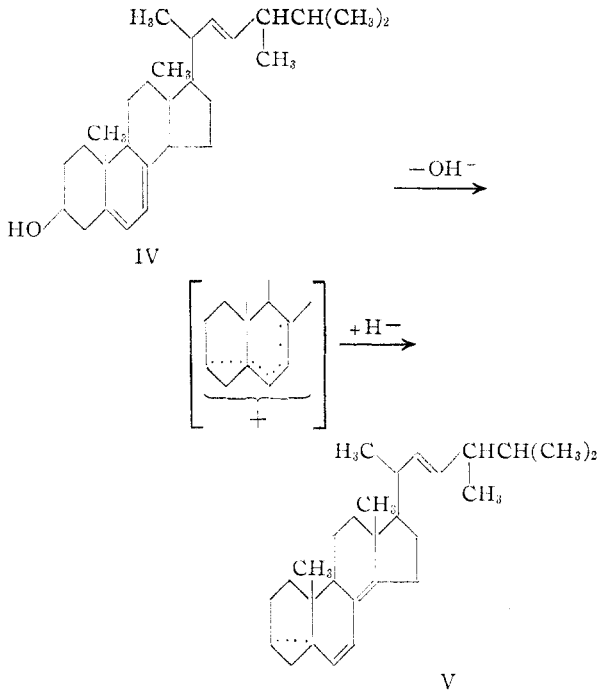
(9) D. H. Gould, K. H. Schaaf and W. L. Ruigh, THIS JOURNAL, **73**, 1263 (1951).

(10) J. Schmutz, H. Schaltegger and M. Sanz, *Helv. Chim. Acta*, **34**, 1111 (1951).

The Göttingen workers did not compare the three isomeric substances by mixed melting point determination and evidently assumed them to be different because of the marked differences in rotation. The similarity in melting point and in ultraviolet absorption suggested to us that they nevertheless are essentially the same hydrocarbon. Repetition of the original preparative procedures gave materials of properties corresponding to those reported. The three preparations show no melting point depressions on admixture and have identical infrared spectra. All three have ultraviolet absorption maxima at 261 mμ, but tetraene B has a notably higher extinction coefficient than A and C. Tetraene B also has the highest melting point and is the least dextrorotatory of the three preparations. We found that both A and C can be further purified by adsorption on heat-activated alumina or by refluxing in amyl alcohol, with resulting increase in melting point and decrease in rotation. The observations suggest that the principal product of dehydration is accompanied by a much more dextrorotatory substance that is rearranged on alumina or by heat treatment to the main product. The impurity must be a hydrocarbon, since analyses of our most dextrorotatory material agreed with the calculated values for $C_{28}H_{42}$. It is attacked preferentially by selenium dioxide, and purification of the main product can be accomplished with 90–95% recovery by brief treatment with this reagent at 0°. Our best sample of purified hydrocarbon had the constants: m.p. 102–102.5°, α_D +92° Chf, λ_{max}^{alc} 261 mμ (26,800). The substance is prepared most conveniently by dehydration with *p*-toluenesulfonyl chloride in pyridine followed by isomerization of the impurity on heat-activated alumina. The hydrocarbon is not reducible with sodium and ethanol. We failed to confirm the statement that the preparation B adds maleic anhydride[3] (non-crystalline product, no analyses).

The Göttingen investigators reported one experimental observation that is incompatible with a tetraene structure for the principal component of their mixtures A, B and C. Although the analyses corresponded to a tetraene structure and hydrocarbon B on perhydrogenation yielded ergostane,[3,11] each of the three preparations on titration with perbenzoic acid absorbed only three equivalents of oxygen. Perbenzoic acid adds to all known types of steroid double bonds, even at the hindered $\Delta^{9(11)}$-position, and the only uncertainty in perbenzoic acid titrations is that the reagent sometimes gives results corresponding to more than the actual number of double bonds in consequence of an initial dehydrogenation to a more extended system of unsaturation.[12] The results thus suggest the presence of three double bonds and another center that is hydrogenable but unreactive to perbenzoic acid.

These requirements correspond to an *i*-steroid structure, as in formula V, for which the systematic name in terms of the proposals of the Ciba conference[13] is 3,5-cyclo-$\Delta^{6,8(14),22}$-ergostatriene. This specific formulation is based in part on consideration of the ultraviolet absorption at 261 mμ. Klotz[14] examined 3,5-cyclo-Δ^6-cholestene (Riegel's "Δ^6-*i*-cholestadiene"[15]) and found that conjugation of the cyclopropane ring with a double bond results in maximal absorption at about 210 mμ, a value intermediate between the maxima for an isolated double bond and for a conjugated diene. An additional double bond at the 8,14-position would be expected to produce a shift of 50 mμ, and hence the maximum calculated for structure V is 260 mμ. An alternate structure having a 6,7;8,9-diene system should absorb at longer wave length and is definitely ruled out by the high extinction coefficient found for the hydrocarbon.



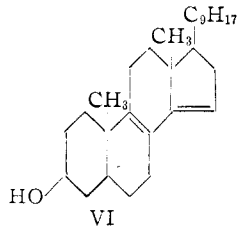
The method of formation by treatment with tosyl chloride in pyridine is consistent with the standard method of preparing an *i*-steroid by rearrangement of the 3-tosylate of a Δ^5-stenol. The standard route was investigated[16] but found less advantageous than the earlier procedures. Ergosteryl tosylate, obtained in not fully pure condition and in rather low yield, is extremely unstable and decomposes readily to the Göttingen hydrocarbon.

An *i*-ergosterol has never been prepared. That the reaction product is a hydrocarbon rather than a hydroxy derivative is evidently because the intermediate is a carbonium ion in which the charge is distributed between positions 3 and 8 and which stabilizes itself by expulsion of a proton. Since formation of an *i*-steroid is a stereospecific reaction,[17] the contrasting behavior of lumisterol[7] is understandable.

Since the *i*-ring of 3,5-cyclo-Δ^6-cholestene does not react with perbenzoic acid but can be opened by hydrogenation,[15] the proposed formulation, (V) is consistent with the reactions of the Göttingen

(11) The reduction product of A was obtained only as an oil.
(12) A. Windaus and A. Luttringhaus, *Ann.*, **481**, 119 (1930).
(13) *Chemistry and Industry*, **25**, 581 (1951).
(14) I. M. Klotz, THIS JOURNAL, **66**, 88 (1944).
(15) B. Riegel, G. P. Hager and B. L. Zenitz, *ibid.*, **69**, 2562 (1946).
(16) Experiments by John C. Babcock.
(17) R. M. Dodson and B. Riegel, *J. Org. Chem.*, **13**, 424 (1948).

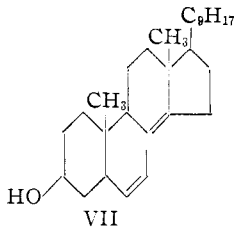
hydrocarbon cited above. Reactions analogous to the acid-catalyzed hydration of 3,5-cyclo-Δ^6-cholestene with reformation of cholesterol and to transformation of the *i*-steroid into cholesteryl chloride[15] have also been reported. Stoll[4] effected hydration of hydrocarbon B with trichloroacetic acid (through the trichloroacetate) and obtained a product, precipitable with digitonin and hydrogenable to $\Delta^{8(14)}$-ergostenol, which was later recognized as a mixture of ergosterol isomers.[18] In view of the lability of the diene system of ergosterol in the presence of acids, it is not surprising that acid-catalyzed hydration is attended with bond migration, particularly since the hydrocarbon is surprisingly stable to hydration. Thus we recovered unchanged starting material after heating the hydrocarbon with 1% aqueous sulfuric acid at 150° for nine hours or after refluxing it with 5% methanolic sulfuric acid for five hours. On repetition on Stoll's experiment we obtained an alcohol mixture showing absorption bands at 280 and 250 mμ indicative of the presence of ergosterol and B-isomers. In our hands hydration was accomplished most satisfactorily by refluxing the hydrocarbon in aqueous acetone containing 10% sulfuric acid. In this case no ergosterol was detected and the product appeared to be a mixture of all three B-isomers (VI–VIII) in which ergosterol-B_1 predominates.



VI

Ergosterol-B_1

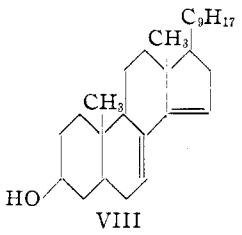
Predicted λ_{max}^{EtOH} 249 (19,800)



VII

Ergosterol-B_2

λ_{max}^{EtOH} 253 (17,000)



VIII

Ergosterol-B_3

λ_{max}^{EtOH} 242 (9,900)

Windaus and co-workers[19] isolated the three B-isomers from mixtures, but present evidence indicated that the final samples were not fully homogeneous. Barton and Brooks[20] found that the B_3-isomer predominates on isomerization with hydrogen chloride at −30° and that pure B_3 can be isolated fairly readily by this method. Pure B_2 has been obtained by sodium–propanol reduction of 14-dehydroergosterol.[21] Pure B_1 has not been reported but the absorption characteristics can be surmised from those of 22-dihydroergosteryl B_1 acetate[20,22] (248 mμ, *E* 19,800) and of the similarly constituted $\Delta^{8,14}$-cholestadienol[23] (250 mμ, log *E* 4.3). The calculated maximum for all three dienes is the same, 244 mμ, and no explanation has been advanced for the deviations from this value of 5 and 9 mμ noted with B_1 and B_2. The low extinction coefficient of B_3 in contrast to the high values for B_1 and B_2 accords with the principal that cisoid chromophores absorb with lower intensity than transoid systems.[24]

The chief component of the mixture that we obtained by hydration of 3,5-cyclo-$\Delta^{6,8(14),22}$-ergostatriene, purified as the acetate by removal of ergosteryl B_3 acetate with maleic anhydride, had constants agreeing fairly closely with those of a sample of B_1 acetate prepared according to Windaus (Table II), although the intensity of absorption at 249 mμ was somewhat lower. Repeated crystallization of the Windaus material was attended with steady increase in melting point and decrease in rotation.

TABLE II

ERGOSTERYL B_1 ACETATE

Method of preparation	M.p., °C.	α_D Chf	λ_{max}^{EtOH} (*E*)
From cycloergostatriene	139–140	−65	249 (13,800)
Windaus	140–141	−54	250 (15,500)
Windaus, ten recrystallizations	148.5–150	−35	250 (16,000)
From "β"-dihydroergosterol	136–137	−46	249 (18,000)

We then attempted an alternate preparation starting with material made (as acetate) by acid isomerization of 5-dihydroergosteryl acetate (IX, preparation[25]) and known both as "β"-dihydroergosterol[26] and as dihydroergosterol III.[27] Barton[28] has presented evidence that the material is an inseparable mixture of the acetates of equal parts of $\Delta^{8(14),22}$-ergostadienol (X) and $\Delta^{14,22}$-ergostadienol (XI). Heilbron, Johnstone and Spring[26] have reported dehydrogenation of "β"-dihydroergosterol with mercuric acetate to an isomer of ergosterol, designated ergosterol E, and since they did not characterize the substance spectrographically we thought that it might contain ergosterol B_1. Repetition of their experiment, however, gave a product of melting point corresponding to theirs (acetate, m.p. 199–120°) that showed feeble absorption at 250 mμ (*E* 150), indicative of the presence of only a trace of conjugated diene. Analysis of our product suggests that the so-called ergosterol E is largely unchanged "β"-dihydroergosterol. We then submitted "β"-dihydroergosteryl acetate to dehydrogenation with selenium dioxide and isolated, after chromatography and in small yield, a product of constants given in the last line of Table II; it melts at a lower temperature

(18) Z. Nakamiya, *Z. physiol. Chem.*, **203**, 255 (1931).

(19) A. Windaus, K. Dithmar, H. Mürke and F. Suckfüll, *Ann.*, **488**, 91 (1931).

(20) D. H. R. Barton and C. J. W. Brooks, *J. Chem. Soc.*, 257 (1951).

(21) D. H. R. Barton and T. Brunn, *ibid.*, 2728 (1951).

(22) D. H. R. Barton and J. D. Cox, *ibid.*, 214 (1949).

(23) W. J. Adams, V. Petrow and R. Royer, *ibid.*, 678 (1951).

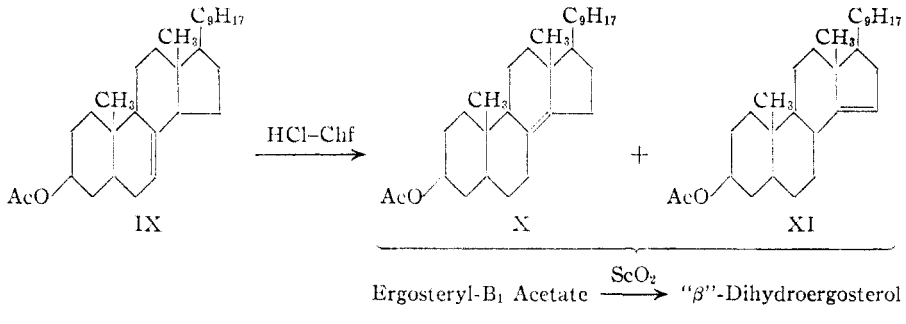
(24) H. P. Koch, *Chemistry and Industry*, **61**, 273 (1942).

(25) D. G. R. Barton and J. D. Cox, *J. Chem. Soc.*, 1354 (1948).

(26) I. M. Heilbron, F. Johnstone and F. S. Spring, *ibid.*, 2248 (1929).

(27) K. Dithmar and Th. Achtermann, *Z. physiol. Chem.*, **205**, 55 (1932).

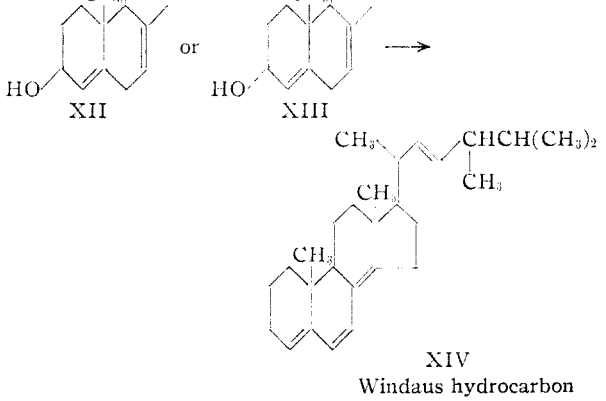
(28) D. H. R. Barton, J. D. Cox and N. J. Holness, *J. Chem. Soc.*, 1771 (1949).



than the other preparations of B_1 acetate melt but has a higher extinction coefficient. The optical rotation has an intermediate value. Since molecular rotation differences are somewhat uncertain in this case, owing to possible vicinal effect of the double bond in the side chain on nuclear double bonds,[29] the extinction coefficient may be a more reliable guide to purity. We are not convinced, however, that any of the samples of B_1 are fully homogeneous. Samples prepared in the three different ways showed no depression in melting point when mixed with one another.

The Göttingen workers reported that the hydrocarbon preparations A, B and C all react with hydrogen chloride in chloroform to give the same crystalline product designated "ergostatetraene hydrochloride."[3,6] Our examination indicates that the material is a mixture of ergosteryl B_1, B_2 and B_3 chlorides. Removal of the B_3 chloride as the maleic anhydride adduct gave us material that we consider to consist mainly of ergosteryl B_1 chloride; λ_{max}^{EtOH} 248 mμ (18,300).

We were unable to isolate the labile, strongly dextrorotatory by-product of the preparation of cycloergostatriene, but considered the possibility that it might be identical with a hydrocarbon of α_D +285° prepared by Windaus[30] by refluxing either alloergosterol (XII) or epialloergosterol (XIII) with methanolic hydrochloric acid; Heilbron later obtained it by high-vacuum sublimation of XIII in the presence of ferric chloride.[31]



Examination of the hydrocarbon showed that it is not the by-product of cycloergostatriene, since it strongly depressed the melting point of our most dextrorotatory material and was not isomerized to the *i*-steroid by activated alumina or by refluxing amyl alcohol. Windaus did not suggest a formula, but Heilbron proposed that the Windaus hydrocarbon is $\Delta^{2,4,7,22}$-ergostatetraene. This structure is ruled out by the absorption characteristics, since the corresponding substance should show maximal absorption around 273 mμ and the *E* should be less than 10,000, whereas the hydrocarbon has a maximum at 283 mμ, *E* 36,000. We believe that the substance is $\Delta^{4,6,8(14),22}$-ergostatetraene (XIV), for which the calculated maximum is 284 mμ and which, having an extended transoid system, should have strong absorption. Supporting evidence is that the Windaus hydrocarbon is converted into $\Delta^{8(14)}$-ergostene by hydrogenation in a neutral medium. It is reducible with sodium and propanol, but the product in this case is an inseparable mixture of trienes one of which shows absorption at 252 mμ.

Dehydration of other $\Delta^{5,7}$-sterols leads to apparently analogous products. 7-Dehydrocholesterol under the same conditions gave a product of absorption maximum at 260 mμ, but the substance could not be obtained crystalline.[32] Dr. Carl Djerassi has informed us of an experiment at Syntex S.A. in which dehydration of 7-dehydrodiosgenin with phosphorus oxychloride–pyridine gave $\Delta^{6,8(14)}$-3,5-cyclo-22a-spirostadiene, $C_{27}H_{38}O_2$, m.p. 170–172°, $\alpha^{22}D$ +108°, λ_{max}^{EtOH} 256 mμ.

Experimental[33]

3,5-Cyclo-$\Delta^{6,8(14)22}$-ergostatriene (V).—When prepared by Rygh's[3] procedure by dehydration with phosphorus oxychloride, material corresponding to "ergostatetraene-A" was obtained in 50% yield; tiny white plates from ether–methanol, m.p. 97–98.5°, α_D +173° Chf, λ_{max}^{EtOH} 261 mμ (17,600); found: C, 88.89; H, 11.19 (see below). Three crystallizations from ether–methanol raised the melting point to 98.8–100° and lowered the α_D to 158° Chf.

Dehydration of ergosterol with *p*-toluenesulfonyl chloride according to Stoll's procedure for preparation of "ergostatetraene-B" furnished the *i*-hydrocarbon in 50–60% yield. It is isolated most readily by chilling the reaction mixture to 0° for several hours, collecting the brownish solid by filtration, washing several times with cold methanol and crystallizing from ether–methanol. Different preparations melted in the same temperature range (100–102°), but the rotation varied from +138 to +115°. A typical sample had the following constants: m.p. 101–102°, α_D +125° Chf, λ_{max}^{EtOH} 260.5 mμ (22,600); found: C, 88.70; H, 11.11. Recrystallizations raised the melting point slightly and lowered the dextrorotation, but we were unable to raise the melting point to 105°, the reported constant for "ergostatetraene-B."

Purification.—Chromatography in the usual manner on acid-washed alumina afforded some separation; early fractions showed somewhat lower dextrorotation than later fractions: after chromatography on heat-activated alumina (heated at 240–250° for eight to ten hours with protection against moisture) most of the eluted material was essen-

(29) D. H. R. Barton and C. J. W. Brooks, THIS JOURNAL, **72**, 1633 (1950).

(30) A. Windaus and K. Buchholz, *Ber.*, **72**, 597 (1939).

(31) J. Barnett, I. M. Heilbron, E. R. H. Jones and K. T. Verill, *J. Chem. Soc.*, 1390 (1940).

(32) Experiment by Josef E. Herz.

(33) All melting points (taken in evacuated capillaries) are uncorrected. All rotations were determined in chloroform at 20–25°. Microanalyses by Mrs. Shirley Golden.

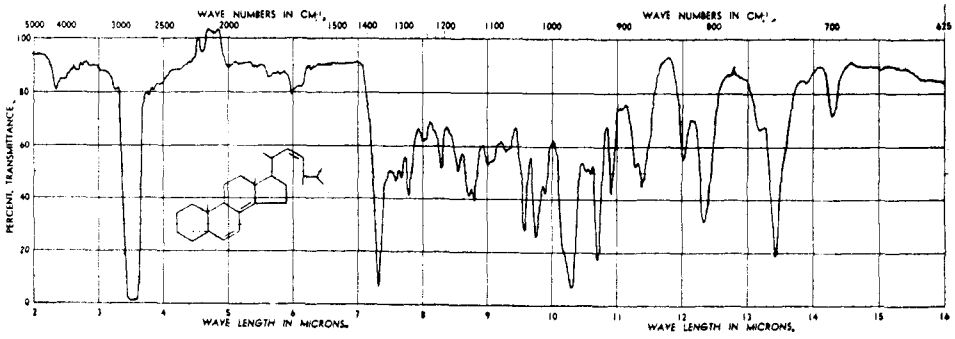


Fig. 1.—Spectrum of 3,5-cyclo-$\Delta^{6,8\,(14),22}$-ergostatriene.

tially pure cycloergostatriene, which after one crystallization from ether–methanol was obtained as rectangular plates, m.p. 102–102.5°, α_D +92° Chf, λ_{max} 261 mμ (26,800).

Purification could be effected even more conveniently by stirring a solution of the dehydration product in petroleum ether with heat-activated alumina for six hours at room temperature (85–90% recovery).

Purification was also effected by treating 560 mg. of crude hydrocarbon, m.p. 100.5–101.5°, α_D +127°, dissolved in 20 cc. of ether and 15 cc. of glacial acetic acid at 0° with 6 cc. of 0.1 *M* selenious acid in acetic acid containing 2% water. After three hours at 0° some red selenium had deposited; the mixture was diluted with water, extracted with ether and the extract was washed with aqueous sodium bicarbonate and filtered through a 3-inch column of Darco to remove selenium. After usual chromatography, pure cycloergostatriene was recovered in 91% yield; m.p. 102–102.5°, α_D +91° Chf, λ_{max}^{EtOH} 261 (25,600).

Equally pure material was obtained by refluxing crude hydrocarbon in redistilled amyl alcohol for five hours (90–95% recovery). This method was used originally to prepare "ergostatetraene C," which, judged by its constants, was still somewhat impure. In our hands this process gave material corresponding to our purified *i*-triene: white elongated thick plates from ether–methanol, m.p. 101–102.5°, α_D +92°, λ_{max} 260.5 mμ (25,800).

Anal. Calcd. for $C_{28}H_{42}$ (378.62): C, 88.82; H, 11.18. Found: C, 88.69; H, 11.19.

The infrared spectrum of cycloergostatriene (Fig. 1) shows many bands in the region associated with absorption by double bonds of the triene system. A band at 9.9 μ is considered to be typical of simple cyclopropane derivatives[34]; but no one band seems to be characteristic of the cyclopropane ring in even simple *i*-steroids.[35] In the case of cycloergostatriene any typical *i*-steroid bands might well be obscured by absorption bands associated with the double bonds.

The hydrocarbon gives a dark amber color which fades to yellow with tetranitromethane, a positive Rosenheim test (initial green color changing to blue), a strongly positive Tortelli–Jaffe test, and a color array with antimony trichloride, red to orange to purple.

Crude Ergosteryl Tosylate.[16]—A mixture of 3 g. of anhydrous ergosterol, 15 cc. of dry pyridine, 5 cc. of dioxane and 4 g. of *p*-toluenesulfonyl chloride was let stand overnight at 25° in the dark and the reaction mixture was diluted with aqueous acetone and chilled overnight. The hard granular product that separated was washed well with dilute acetone; yield 1.0 g., m.p. 116° dec. Recrystallization from ethyl acetate on ligroin did not raise the m.p.; λ_{max}^{chf} 274, 284, 294 mμ (log *E* 4.03, 4.03, 3.81); 6.25, 7.37, 8.55 μ; $\alpha^{27}D$ −45° Chf. The analysis is in only approximate agreement with the theory.

Anal. Calcd. for $C_{35}H_{50}O_3S$ (550.81): C, 76.31; H, 9.15. Found: C, 75.84; H, 9.53.

Dilution of the reaction mixture mother liquor afforded 1.5 g. of crude cycloergostatriene, which, after several recrystallizations from ethyl acetate–methanol, had the constants m.p. 93–100°, α_D +157.3° Chf. In an attempt to determine the ultraviolet absorption of the tosylate in ethanol, the sparingly soluble substance had to be warmed with the solvent for several minutes to effect solution; this treatment evidently effected cleavage, since the three-banded spectrum was replaced by a single band at 260 mμ (16,300).

Ergosteryl B_1 Chloride.—Dry hydrogen chloride was bubbled into an ice-cold solution of 0.5 g. of cycloergostatriene in 50 cc. of chloroform for two hours. The solution was washed until neutral with bicarbonate, dried and evaporated under reduced pressure. The yellow residue was heated with maleic anhydride in refluxing benzene for eight hours to remove B_3 chloride and then crystallized from ethanol or acetic anhydride: colorless leaflets, m.p. 117–118°, α_D −30° Chf, λ_{max}^{EtOH} 248 mμ (18,300); positive Beilstein test.

Anal. Calcd. for $C_{28}H_{43}Cl$ (415.08): C, 81.01; H, 10.44. Found: C, 80.89; H, 10.59.

Ergosterol B_1. (a) Hydration of Cycloergostatriene with Trichloroacetic Acid.—The hydrocarbon was treated with trichloroacetic acid according to Stoll[4] and the product heated with Claisen alkali for 2 hr. on the steam-bath, acetylated and chromatographed. A white solid eluted by petroleum ether on crystallization from benzene–ethanol gave white plates, m.p. 127.5–128°, α_D −106° Chf, λ_{max}^{EtOH} 251 mμ (11,400), 281 mμ (2,480).

Anal. Calcd. for $C_{30}H_{46}O_2$ (438.67): C, 82.13; H, 10.57. Found: C, 82.29; H, 10.71.

The absorption at 281 mμ indicated the presence of some ergosteryl acetate; two further crystallizations effected little change: m.p. 136–137°, α_D −98° Chf, λ_{max}^{EtOH} 251.5 mμ (12,200), 281.5 mμ (4,740).

In another experiment the hydrolyzed product from 12 g. of hydrocarbon was refluxed for 3 hr. with 500 cc. of 5% ethanolic hydrochloric acid to isomerize any ergosterol present. The product (10 g.), after acetylation, chromatography and two crystallizations from ether–methanol formed elongated rectagonal prisms, m.p. 143–144°, α_D −60° Chf, λ_{max}^{EtOH} 250 mμ (12,300).

Anal. Calcd. for $C_{30}H_{46}O_2$ (438.67): C, 82.13; H, 10.57. Found: C, 82.32; H, 10.33.

The free sterol (B_1) crystallized from methanol in rectangular prisms, m.p. 144–145°, α_D −47° Chf, λ_{max}^{EtOH} 250 mμ (10,900).

Anal. Calcd. for $C_{28}H_{44}O \cdot {}^1/_2CH_3OH$ (412.65): C, 82.95; H, 11.24. Found: C, 82.98; H, 11.00.

Windaus'[19] preparation of ergosterol B_1 melted at 148°, α_D −40° Chf.

(b) **Hydrolysis with Sulfuric Acid.**—An ice-cold solution of 15 g. of cycloergostatriene in 800 cc. of acetone and 40 cc. of water was treated gradually with 95 cc. of 96% sulfuric acid and the yellow-green solution refluxed under nitrogen for 3 hr., when it had become dark brown. Evaporation of the acetone left 139 g. of dark oil consisting mainly of condensation products of acetone; after adsorption on 500 g. of alumina, elution with petroleum ether removed most of the acetone products as a sweet-smelling brown oil (120 g.). Elution with benzene–ether (2:1) then gave 5 g. of reddish solid, which when chromatographed as the acetate afforded 3 g. of light yellow solid (eluted by petroleum ether). Crystallization from ether–methanol then gave colorless prisms, m.p. 136–137°, α_D −67° Chf. This material was refluxed with maleic anhydride in benzene for 4 hr. and the solution diluted with petroleum ether and chromatographed

(34) J. M. Derfer, E. E. Pickett and C. E. Boord, THIS JOURNAL, **71**, 2482 (1949); R. V. Volkenburgh, K. W. Greenlee, J. M. Derfer and C. E. Boord, *ibid.*, **71**, 3595 (1949).

(35) M. L. Josien, N. Fuson and A. S. Carey, *ibid.*, **73**, 4445 (1941).

to remove maleic anhydride and B_3-adduct. Crystallization from ether–methanol then gave prisms, m.p. 139–140°, α_D −65° Chf, λ_{max}^{EtOH} 249 mμ (13,800).

Anal. Calcd. for $C_{30}H_{46}O_2$ (438.67): C, 82.13; H, 10.57. Found: C, 81.94; H, 10.36.

Hydrolysis with Claisen alkali (40 min. on steam-bath) and crystallization from methanol gave ergosterol B_1 as prisms, m.p. 141.5–143°, α_D −52° Chf, λ_{max}^{EtOH} 249.5 mμ (12,800).

Anal. Calcd. for $C_{28}H_{44}O \cdot {}^1/_2CH_3OH$ (412.65): C, 82.95; H, 11.24. Found: C, 83.13; H, 11.20.

Mixtures of the alcohol and acetate with samples of ergosterol B_1 and its acetate described in the next section showed no depression in m.p.

(c) **By the Windaus Procedure.**[19]—After hydrogen chloride had been passed into an ice-cold solution of 5 g. of ergosteryl acetate in 50 cc. of chloroform (containing 0.75% ethanol) for 2 hr., the violet solution was concentrated to one-half on the steam-bath and the remaining solvent was removed in vacuum. Crystallization gave a tan solid (2.1 g.) from which the B_3-acetate was removed by treatment with maleic anhydride and chromatography, which afforded 650 mg. of ergosterol B_1 acetate and 870 mg. of free B_1, probably resulting from alcoholysis by the ethanol in the commercial chloroform. The acetate formed plates, m.p. 134.2–135.2°, from ether–methanol, α_D −57° Chf (found: C, 82.23; H, 10.46); the alcohol (methanol), m.p. 140.5–141°, α_D −39° Chf (found: C, 82.93; H, 11.23). The alcohol and the acetate both gave Tortelli–Jaffe and Rosenheim color tests and a yellow to red color with antimony trichloride; they did not react with selenium dioxide in acetic acid at 25°.

Another batch of B_1 acetate (9.2 g.) from 30 g. of ergosteryl acetate was processed a second time with maleic anhydride, which raised the m.p. to 140–141°, α_D −54° Chf, λ_{max}^{EtOH} 250 mμ (15,500). Ten crystallizations from various solvents, particularly ether–methanol, gradually raised the m.p. to 148.5–150° and lowered the rotation to −36° Chf, λ_{max}^{EtOH} 250 mμ (16,000); found: C, 81.96; H, 10.77. There were no indications of an approach to constancy.

(d) **Isomerization of Ergosterol with Ethanolic Hydrochloric Acid.**[36]—A solution of 2 g. of ergosterol in 175 cc. of 95% ethanol and 10 cc. of 36% hydrochloric acid was refluxed for 3 hr. and the solvent was removed without cooling, first at normal pressure and then at 20 mm. The residue was dissolved in 15 cc. of pyridine and the filtered solution treated with an equal volume of acetic anhydride and heated on the steam-bath for ½ hr. Water was added dropwise to the point of saturation, and the chilled mixture afforded 1.5 g. of solid product (washed with methanol). Crystallization from ethyl acetate–methanol (Darco) and then from ether–methanol gave colorless prisms, m.p. 143.5–144°, α_D −49° Chf, λ_{max}^{EtOH} 251 mμ (15,300); found: C, 82.40; H, 10.36.

The process is much simpler than any of the others and gives material comparable to that of (c). It can be applied equally well to ergosteryl acetate.

(e) **By Dehydrogenation of "β"-Dihydroergosteryl Acetate.**—5-Dihydroergosteryl acetate was prepared by the method of Barton and Cox[25] and purified by heating a benzene–acetone solution with potassium permanganate, followed by chromatography; crystallized from chloroform–methanol, it melted at 180–181°, α_D −18° Chf. Isomerization with hydrogen chloride in chloroform was conducted according to Heilbron[26]; since the process was attended with some deacetylation, the product was reacetylated and crystallized from ether–methanol. The resulting "β"-dihydroergosteryl acetate formed thin prismatic needles, m.p. 104–105.5°, α_D −17° Chf.

Dehydrogenation of 1.5 g. of the corresponding alcohol (m.p. 112–113°) with mercuric acetate in ethanol according to Heilbron[28] was attended with separation of only a fourth of the theoretical amount of mercurous acetate, and purification of the product by acetylation, treatment with maleic anhydride in benzene solution (5 hr. reflux), chromatography, and crystallization afforded 1.1 g. of material corresponding to Heilbron's "ergosteryl E acetate," of the following properties: m.p. 117–118°, α_D −21° Chf, λ_{max}^{EtOH} 250 mμ (150). The extinction coefficient indicates that very little conjugated diene is present, and the composition is closer to that of the starting material than to that of a product of dehydrogenation.

Anal. Calcd. for $C_{30}H_{48}O_2$: C, 81.76; H, 10.98. For $C_{30}H_{46}O_2$: C, 82.13; H, 10.57. Found: C, 82.16; H, 10.73.

Hydrolysis and crystallization from ether–methanol gave colorless needles, m.p. 124–125.5°, that also appeared to be largely unchanged "β"-dihydroergosterol. Attempted dehydrogenation of "β"-dihydroergosteryl acetate with mercuric acetate likewise gave an acetate (m.p. 117–118°, α_D −19° Chf) and alcohol (m.p. 123–124°, α_D −13° Chf; found: C, 84.71; H, 11.46) corresponding to the supposed ergosteryl E acetate and alcohol but apparently consisting essentially of starting material.

"β"-Dihydroergosteryl acetate was then treated in benzene solution at 25° with a solution of two equivalents of selenious acid in acetic acid containing 2% of water. The solution turned yellow in 1–2 minutes and red selenium started precipitating in 5–10 minutes. After standing overnight, the mixture was diluted with water and extracted with ether; the ethereal extract was filtered through Darco to remove colloidal selenium and the colorless filtrate diluted with methanol, concentrated to the point of saturation and set aside for crystallization. Ergosterol B_1 acetate separated on standing in 10–20% yield: m.p. 136–137°, α_D −46° Chf, λ_{max}^{EtOH} 249 mμ (18,100); no depression with above samples (found: C, 81.94; H, 10.62). Saponification and crystallization from methanol gave the free alcohol, m.p. 142°, α_D −41° Chf, λ_{max}^{EtOH} 250 mμ (19,400); found: C, 83.67; H, 10.71.

The Windaus Hydrocarbon[30]: **$\Delta^{4,6,8(14),22}$-Ergostatetraene (XIV).**—The starting material, ergosterone ($\Delta^{4,7,22}$-ergostatrienone), was prepared by oxidation of 50 g. of ergosterol according to Oppenauer.[37] A chromatograph of the reaction mixture afforded 30.4 g. of crude ergosterone, eluted by 1:1 petroleum ether–benzene, and 19.4 g. of crude ergosterol, eluted by 1:1 benzene–ether. Rechromatography yielded 25 g. (50%) of ergosterone, m.p. 128–130°, and two crystallizations from methanol, which proved to be a much better solvent than acetone–petroleum ether, gave fine, faintly yellow-green needles, m.p. 131.5–132°, α_D −10° Chf, λ_{max}^{EtOH} 238 mμ (17,000), corresponding to previous reports.[30,37,38]

Meerwein–Ponndorf reduction of 10 g. of ergosterone, m.p. 128–130°, and processing according to Marker[39] gave 8.5 g. of a mixture of alloergosterol and epialloergosterol as a yellowish solid melting over a range up to 164° dec. A suspension of 8.0 g. of the mixture in 1.1 l. of boiling methanol was treated with 2.6 cc. of 36% hydrochloric acid and the resulting solution refluxed for 1 hr. On standing overnight at 25° a little tar separated and was removed; evaporation of the filtrate left a brown residue that was leached repeatedly with petroleum ether for chromatography. Elution with the same solvent gave 3.8 g. of yellowish solid that on two crystallizations from ether–methanol afforded 1.8 g. of the ergostatetraene as white plates, m.p. 86–87°, α_D +283° Chf, λ_{max}^{EtOH} 283 mμ (33,000).

Anal. Calcd. for $C_{28}H_{42}$ (378.62): C, 88.82; H, 11.18. Found: C, 88.96; H, 11.12; mol. wt., 350.[40]

The hydrocarbon slowly turns yellow on storage at 25° but is stable at 0–5°. It does not react with selenium dioxide in acetic acid at 25°, but gives positive Tortelli–Jaffe, Rosenheim and antimony trichloride tests. With tetranitromethane an initial amber color changes to yellow in about one minute.

Material of the same properties was obtained from the epimer mixture (m.p. 158–162°) resulting from reduction of ergosterone with lithium aluminum hydride. Processing of the mixture with digitonin afforded crude alloergosterol, m.p. 119–121°, and crude epialloergosterol, m.p. 146–148° after chromatography, in yields of 35–50% and 20–30%. Each epimer afforded the Windaus hydrocarbon with substantially the same result as when a mixture was used.

(36) Based upon a procedure applied to 7-dehydrocholesterol, Guy H. Ourisson, Dissertation, Harvard University, 1952.

(37) R. V. Oppenauer, *Rec. trav. chim.*, **56**, 137 (1937).

(38) F. Wetter and K. Dimroth, *Ber.*, **70**, 1665 (1937).

(39) R. E. Marker, O. Kamm, J. F. Laucius and T. S. Oakwood, THIS JOURNAL, **59**, 1840 (1937).

(40) Micro Rast determination by Koji Nakanishi.

A solution of 110 mg. of $\Delta^{4,6,8(14),22}$-ergostatetraene in ethyl acetate in the presence of used platinum catalyst absorbed three moles of hydrogen in 30–40 min. and the reaction then stopped. The resulting colorless oil slowly crystallized from ether–methanol at 5° to give 80 mg. of crystals, m.p. 71–74°, α_D +16° Chf. Recrystallized material melted at 74.5–75.5°, α_D +15° Chf; positive test with tetranitromethane. The constants reported[41] for $\Delta^{8(14)}$-ergostene are: m.p. 77–78°, α_D +11°.

The tetraene (110 mg.) was reduced when sodium (4 g.) was added over a 4-hr. period to a refluxing solution in redistilled *n*-propyl alcohol (25 cc.). The solution was let stand overnight, ether was added and the cake broken up, and then successive small portions of methanol were added to decompose the sodium. The washed, dried, and clarified ethereal solution on concentration (3–4 cc.) and cooling (5°) afforded two crops of product: 46 mg. of long thick plates, m.p. 68.5–69.5°, α_D −124° Chf, λ_{max}^{EtOH} 252 mμ (4,600); 33 mg., m.p. 58–59.5°, λ_{max}^{EtOH} 251.5 mμ. The material is probably a mixture, since in known instances chemical reduction of trienes involve 1,2-, 1,4- and 1,6-addition.

(41) I. M. Heilbron, F. S. Spring and E. T. Webster, *J. Chem. Soc.*, 1705 (1932).

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[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES[a] AND THE NATIONAL INSTITUTE FOR MEDICAL RESEARCH[b]]

The Formation of 1,3,5-Triazines by the Reaction of α-Cyanocarbonyl Compounds with Guanidine

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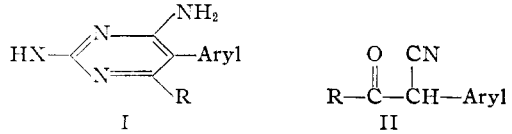
RECEIVED MAY 28, 1952

Contrary to reports in the literature it has been shown that the condensation of cyandesoxybenzoin and guanidine does not result in 2,4-diamino-5,6-diphenylpyrimidine but gives 2-amino-4-benzyl-6-phenyl-1,3,5-triazine. In a similar manner α-formylphenyl- and *p*-chlorophenylacetonitrile condense with guanidine to give 2-amino-4-benzyl- and 4-*p*-chlorobenzyltriazines. These compounds were synthesized by an alternate route. The courses of the above reactions are discussed.

The condensation of an α-cyanocarbonyl compound with a urea derivative would appear to be an attractive method of preparation of 4-aminopyrimidines. This is especially the case with 4-amino-5-arylpyrimidines (I), since the required α-acylarylacetonitriles (II) are readily available by the acylation of arylacetonitriles with esters. However, there appears to be only one instance of such a synthesis reported in the literature. Zerweck and Keller[1] state that the reaction of cyandesoxybenzoin (III) with guanidine yielded 2,4-diamino-5,6-diphenylpyrimidine (IV). All attempts to prepare IV from these two compounds failed and IV, prepared by the condensation of α,β-diphenyl-β-methoxyacrylonitrile and guanidine,[2] did not correspond in properties with the compound described by Zerweck and Keller.

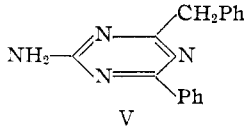
When cyandesoxybenzoin and guanidine were refluxed together in alcoholic solution no product was isolated and the greater part of the ketonitrile was recovered unchanged. However, when the heating was carried out at 180° the solvent being allowed to evaporate, a crystalline product $C_{16}H_{14}N_4$, isomeric with IV, was obtained and the oily residues from the reaction had the odor of ethyl benzoate. The ultraviolet spectrum of the product (Table I) suggested that it was a phenyl-1,3,5-triazine and it was formulated as 2-amino-4-benzyl-6-phenyl-1,3,5-triazine (V).



I

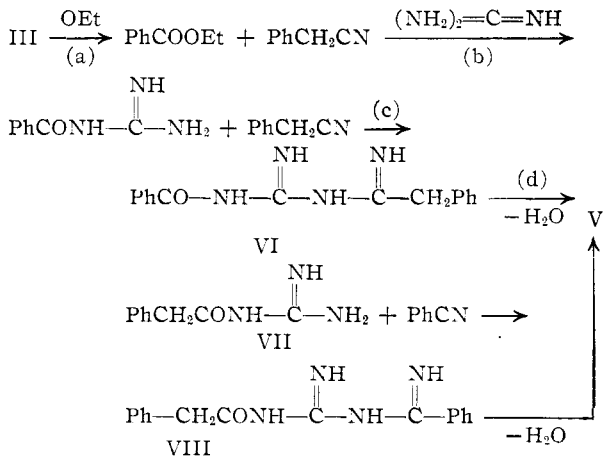
II

IV (R = Aryl = Ph, X = NH) III (R = Aryl = Ph)



V

The formation of V was believed to occur by the following steps: (a) the cleavage of III to ethyl benzoate and phenylacetonitrile by the strongly alkaline solution, a reversal of the formation of III; (b) the reaction of guanidine with ethyl benzoate to give benzoylguanidine; (c) the addition of benzoylguanidine to phenylacetonitrile to give N′-phenyliminoaceto-N″-benzoylguanidine (VI) followed by (d) the cyclization of this compound with loss of water to give V.



If this formulation is correct the condensation of benzoylguanidine and phenylacetonitrile at 180° should give rise to V and further phenylacetylguanidine (VII) and benzonitrile might be expected to give the same product *via* the intermediate N′-benzoylimino-N″-phenylacetylguanidine (VIII). In fact, both these reactions gave rise to V in good yield, thus giving strong support to the assigned structure and mode of formation.

(1) W. Zerweck and K. Keller, U. S. Patent 2,211,710.

(2) P. B. Russell and G. H. Hitchings, THIS JOURNAL, **73**, 3763 (1951).