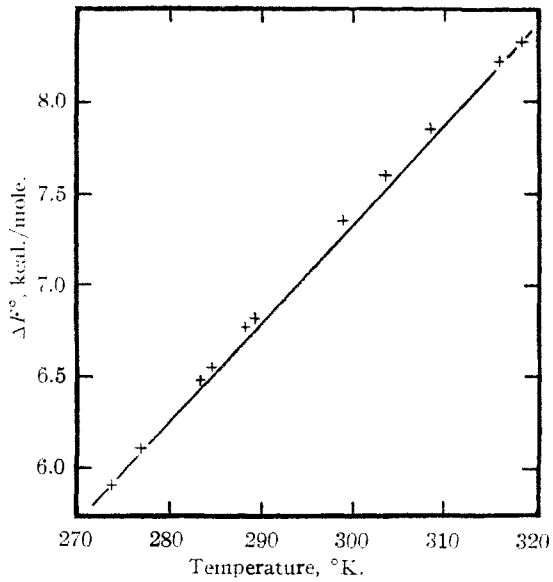

Fig. 1.—Free energy of hydration of toluene in water, ΔF°, plotted against temperature, T, in degrees absolute.

for all of the lower liquid aromatic hydrocarbons as a group.

Thus, the two ways of handling the solubility data, which ways differ in the selection of the standard states of the components, give identical results. It is therefore felt that the conclusions reached by Bohon and Claussen still form a good working hypothesis for further research.

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CORNING, NEW YORK

The Formation of Steroid Azines by Reaction of Ketosteroids with Girard Reagents[1]

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The introduction of Girard reagents T and P for the separation of ketosteroids,[4] has greatly facilitated the isolation of estrone as well as other ketosteroids from biological sources. Since these reagents are widely employed, it will be of interest to report that during the reaction of these hydrazides, especially reagent P, with steroid ketones small amounts of azines are simultaneously formed. These azines in contrast to the usual Girard complexes are not water soluble and therefore appear in the non-ketonic fraction.

In the course of the commercial isolation of ketonic estrogens from pregnant mares' urine, the phenolic fraction is separated into ketonic and non-ketonic components by means of Girard reagent P. During one such separation a crystalline precipitate was formed in an alcoholic solution of the non-ketonic fraction resulting from the treatment of a urinary, phenolic fraction with reagent P. The crystalline material was isolated and identified as estrone azine (I).

That azines may be formed during the treatment of aldehydes with Girard reagents T or P has already been noted by Lederer,[5] who reported that aldazines are produced in 3–33% yield when various aromatic aldehydes are treated with these reagents. Assuming that the azines were formed because of the presence of free hydrazine in the Girard reagent, Lederer considered it possible to avoid this side reaction by using reagent free of the base. Miescher and Schmidlin[6] isolated an unsaturated aldazine (of β,β-diphenylacrolein) during the Girard separation of the ketones produced by oxidation of $\Delta^{4,20,23}$-3-keto-24,24-diphenylcholatriene. They assumed that an unusual splitting of the Girard complex during the hydrolysis step resulted in the formation of the azine instead of the expected carbonyl compound.

In order to study further the formation of estrone azine during the isolation of urinary estrogens by reagent P several model experiments were performed. After refluxing an ethanolic solution of crystalline estrone with the reagent for two hours, 6% of estrone azine was isolated from the non-ketonic fraction. The yield of azine was not decreased by using a freshly recrystallized sample of the reagent which gave a negative test for free hydrazine with the Pesez reagent.[7] The yield of azine was increased to 36% when the time of reflux was lengthened to 24 hours. These results indicate that free hydrazine in the reagent was not responsible for the formation of the azine and suggests that the hydrazine arises by decomposition of the excess reagent. This suggestion was confirmed by refluxing an acetic acid–ethanolic solution of reagent P alone for 24 hours. At the end of this period estrone was added and the heating continued for two additional hours. The yield of azine found in the non-ketonic fraction was 36%. The expected yield of azine formed when estrone and reagent P are refluxed together for two hours is 6–7%.

It can be shown that the formation of the azine is not due to an unusual mode of acid cleavage of the Girard–estrone complex because the azine will precipitate directly from the cooled reaction mixture before either water or acid is added. Addition of water to the reaction mixture did not appear to alter the yield of azine.

These model experiments suggest that the azines are formed by the action of hydrazine arising from the excess reagent P by hydrolysis or alcoholysis. The hydrazine so liberated can then react either with the ketone or react directly with the complex to displace the Girard hydrazide yielding the hydrazone. Under acidic conditions hydrazones are known to be rapidly converted to azines.[8]

It has also been found that other ketosteroids

(1) Presented at the Meeting of the American Chemical Society at Chicago, Ill., September, 1950, Abstracts page 29C.

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such as dehydroisoandrosterone, Δ^5-pregnenolone and testosterone yielded 5–10% of the corresponding azine when treated with Girard reagent P in the customary manner.

Substitution of reagent T for P results in diminished yields of azine. After treating estrone with reagent T for two hours no estrone azine was found in the non-ketonic fraction and after 24 hours, 10% was obtained.

Experimental[9]

Isolation of Estrone Azine (I) from the Non-ketonic Phenolic Fraction of Pregnant Mares' Urine.—A phenolic fraction weighing 41 g. was prepared from pregnant mares' urine by a modification of the Cohen–Marrian[10] procedure. The tar was dissolved in 140 cc. of ethanol containing 14 cc. of acetic acid and treated with 25 g. of Girard reagent P. After refluxing for two hours the solution was poured onto ice and water containing sufficient sodium hydroxide to neutralize the acetic acid. The non-ketonic fraction, extractable with ether, consisted of 35 g. of a black, tarry material. This residue was dissolved in ethanol and stored in the refrigerator. The next day the estrone azine (I) had crystallized from the solution. After several recrystallizations from large volumes of ethanol, the azine melted at 290–300° with decomposition leaving a red melt; $[\alpha]^{25}D$ +48.5 ± 2° (9.30 mg. dissolved in 2.00 cc. of dioxane).

I was difficultly soluble in methanol, acetone and ether but was readily soluble in pyridine and dioxane. Fluorimetric analysis[11] of the azine gave a value equal to 95% that of estrone. The modified Kober reaction[12] gave a color intensity equal to 94% that given by estrone. The Zimmermann reaction[13] was negative. The compound possessed an ultraviolet spectrum identical with that of estrone (maximum at 280 mμ, $E^{1\%}_{1\ cm.}$ 82). There was no evidence of a contribution to the ultraviolet spectrum by an azine chromatophore.[14] Infrared spectral analysis[15] indicated the absence of a free carbonyl group and the presence of phenolic hydroxyl group. Moreover the spectrum contained a band at 6.05 μ which probably is associated with the C=N group.[16]

Biological assay[17] revealed that the substance was estrogenically inactive in doses of at least twenty times that of estrone.

Diacetate of I.—Five hundred mg. of the azine was heated in 3 cc. of acetic anhydride containing 1 cc. of dry pyridine for one hour. Although by this time some of the product had crystallized out of the boiling solution, the mixture was poured onto ice and water and the product extracted into chloroform. The extract was washed with dilute sulfuric acid solution, dilute sodium carbonate solution and water until neutral. Evaporation of the chloroform and recrystallization of the solid residue from chloroform–methanol (or ethyl acetate) gave 281 mg. of the diacetate (II), m.p. 270–273°, $[\alpha]^{32}D$ + 51.2 ± 5° (2.54 mg. dissolved in 2.00 cc. of chloroform).

Anal. Calcd. for $C_{40}H_{48}O_4N_2$: C, 77.38; H, 7.79; N, 4.52; acetyl, 13.5. Found: C, 77.51, 77.66; H, 7.95, 7.46; N, 4.47; acetyl, 13.8.

A second crop of crystals was obtained from the mother liquor, 131 mg., m.p. 269–271°.

Alkaline Saponification of the Diacetate (II).—One hundred forty-five mg. of II was heated in 1 cc. of 5% methanolic potassium hydroxide. Within a few minutes the insoluble compound dissolved and after one hour the solution was poured onto ice and water. The product was filtered and recrystallized twice from ethyl acetate–methanol. It melted at 293–296° (red melt) and did not depress the m.p. of estrone azine.

When I (500 mg.) was refluxed in 5% methanolic potassium hydroxide (25 cc.) for 3.5 hours, it was recovered unchanged, m.p. 293–303°.

Acid Hydrolysis of I.—Twenty-two cc. of 50% sulfuric acid was added to 500 mg. of I suspended in 200 cc. of boiling methanol. In a few minutes the insoluble material dissolved and after heating one hour the clear solution was poured onto ice. The tan precipitate was filtered and recrystallized from methanol; it melted at 251–253°. This was identified as estrone by mixed m.p. determination.

Acid Hydrolysis of Diacetate (II).—Ten cc. of 5% sulfuric acid was added to 20 mg. of II dissolved in 5 cc. of dioxane. The mixture was heated for 20 minutes after which it was diluted with water. The product which precipitated was recrystallized from ethanol and melted at 253°. It did not depress the m.p. of estrone. A Zimmermann test gave a result quantitatively identical with that given by estrone. The infrared spectrum confirmed its identity.

Treatment of I with 2,4-Dinitrophenylhydrazine.—Twenty mg. of I was heated with 25 mg. of 2,4-dinitrophenylhydrazine in 15 cc. of ethanol containing 0.06 cc. of concentrated HCl for three hours. Concentration of the solution resulted in the precipitation of the hydrazone which melted, after one recrystallization from dilute methanol, at 284–285°, log $E_{365\ m\mu}$ 4.49. Admixture with an authentic sample of the 2,4-dinitrophenylhydrazone of estrone did not depress the melting point.

Anal. Calcd. for $C_{24}H_{26}O_5N_4$: C, 64.05; H, 5.82; N, 12.45. Found: C, 63.33; H, 6.11; N, 12.51.

The facile conversion of azines to the corresponding oximes and hydrazones by treatment with the appropriate reagent has been reported by Knopfer.[18]

Treatment of I with Hydroxylamine.—Forty-five mg. of I was refluxed for five hours with 50 mg. of hydroxylamine hydrochloride in ethanol–acetic acid (9:1) solution. The mixture was diluted with water and the precipitate removed by centrifugation. It was recrystallized from ethanol and melted at 233–235°. There was no depression when mixed with an authentic sample of estrone oxime. Fifteen mg. of the oxime was hydrolyzed by heating in 3 cc. of ethanol containing 3 cc. of 10% sulfuric acid solution for three hours. The product was identified as estrone by melting point, mixed melting point, and biological activity.

Preparation of Estrone Azine.—Three hundred mg. of estrone was refluxed for two hours with 1 cc. of 85% hydrazine hydrate in 20 cc. of ethanol–acetic acid (9:1) solution. The reaction mixture was poured onto ice and water containing sufficient sodium hydroxide to neutralize the acetic acid. The precipitate was extracted with ether, the extract washed with water several times and then the ether was evaporated. The azine was crystallized from ethyl acetate and melted at 295–300° with decomposition. It did not depress the m.p. of the estrone azine (I) isolated from the non-ketonic phenolic extracts from pregnant mares' urine. The infrared spectra of the two samples were identical.

Formation of Estrone Azine by Reaction of Estrone with Girard Reagent P.—Three hundred mg. of estrone (m.p. 254°) dissolved in 20 cc. of ethanol–acetic acid (9:1) solution was treated with 500 mg. of reagent P. The mixture was refluxed for two hours and then poured into 180 cc. of ice water containing sufficient sodium hydroxide to neutralize the acetic acid. The precipitate was extracted into ether and the extract, containing the non-ketonic substance, was washed with 10% sodium carbonate solution and with water. The solvent was evaporated and the residue recrystallized from ethyl acetate–methanol. The azine (18 mg., 6%) melted at 285–290° (dec.) and did not depress the m.p. of the sample of estrone azine prepared from estrone and hydrazine hydrate. The infrared spectra of the two samples were identical.

When the reaction was carried out for 24 hours, the yield of azine was increased to 31%. The product melted at 285–290° (dec.) and formed a diacetate which melted at 270–275°.

Anal. Calcd. for $C_{40}H_{48}O_4N_2$: C, 77.38; H, 7.79; N, 4.52. Found: C, 77.39; H, 7.74; N, 4.47.

(9) The microanalyses reported herein were performed by Mr. J. Alicino of the Squibb Institute for Medical Research. Melting points were determined in a Hershberg melting point apparatus or on a Kofler micro melting point block, and are correct to about ±1°.

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(15) We are indebted to Dr. N. Coy of the Squibb Institute for Medical Research for determining and interpreting the infrared and ultraviolet spectra reported in this paper.

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Four hundred mg. of reagent P was dissolved in 20 cc. of ethanol–acetic acid (9:1) solution and the mixture refluxed for 24 hours. At the end of this period 200 mg. of estrone was added and the heating continued for two additional hours. Following the usual work-up, the non-ketonic fraction yielded, after one recrystallization from dilute ethanol, 73 mg. (36.5%) of estrone azine, m.p. 290–300° (dec.).

When Girard reagent T was used instead of reagent P and the reaction carried out as described above for two hours, no azine was formed; however, upon prolonging the heating period for 24 hours, a 10% yield of azine was obtained.

The Formation of Other Steroid Azines during Girard Separation. Dehydroisoandrosterone Acetate.—350 mg. of dehydroisoandrosterone acetate was refluxed with 700 mg. of Girard reagent P in acetic acid–ethanol solution for two hours. After the customary work-up, 25 mg. of the crystalline azine melting at 265–268° was obtained in the non-ketonic fraction. The sample was prepared for analysis by sublimation in high vacuum at 250–275°.

Anal. Calcd. for $C_{42}H_{60}O_4N_2$: C, 76.80; H, 9.21; N, 4.27. Found: C, 77.52; H, 9.05; N, 4.25.

The **azine of dehydroisoandrosterone** was prepared in a similar way and melted at 256°. Acetylation with acetic anhydride and pyridine gave the same diacetoxy derivative, m.p. 265°, as described above.

Pregnenolone.—500 mg. of Δ^5-pregnen-3β-ol-20-one was treated with Girard reagent P (1 g.) in the usual way for two hours. The crystalline azine found in the non-ketonic fraction weighed 40 mg. (96 mg. of azine was obtained when the reaction was carried out for 24 hours). The sample was sublimed in high vacuum at 250–300° and then recrystallized from ethyl acetate–acetone, m.p. 290–295°.

Anal. Calcd. for $C_{42}H_{64}O_2N_2$: C, 80.20; H, 10.26; N, 4.45. Found: C, 80.05; H, 10.57; N, 4.65.

A sample of pregnenolone azine, prepared from pregnenolone and hydrazine hydrate, did not depress the melting point of the above product. Both samples yielded the same diacetoxy derivative, m.p. 253–257° (Kofler block) (plates from benzene–ligroin).

Testosterone.—548 mg. of testosterone was treated for 48 hours with 1 g. of reagent P as described above. Two hundred mg. of the yellow azine was obtained in the non-ketonic fraction. It was recrystallized twice from chloroform–methanol, m.p. 257.5–260° (dec.). For analysis it was dried at 100° for 16 hours.

Anal. Calcd. for $C_{38}H_{56}O_2N_2$: C, 79.68; H, 9.85; N, 4.89. Found: C, 79.64; H, 10.01; N, 5.25.

Its ultraviolet spectrum had two maxima, one at 262 mμ (log E 4.41) and the other at 300 mμ (log E 4.40). A sample of testosterone azine prepared by the reaction of testosterone with hydrazine hydrate exhibited the same ultraviolet spectrum, log $E_{262\ m\mu}$ 4.41 and log $E_{300\ m\mu}$ 4.42. It did not depress the melting point of the product obtained from the reaction of testosterone with Girard reagent P.

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Acetylation of 1-Methylcyclohexene

BY N. C. DENO AND HARRY CHAFETZ

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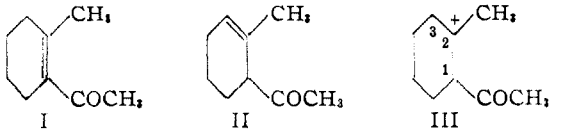
Yields of about 50% have frequently been reported for the stannic chloride catalyzed acetylation of 1-methylcyclohexene with acetyl chloride. However, the product obtained after the customary alkaline treatment is an equilibrium mixture of 1-methyl-2-acetylcyclohexene (I) and 1-methyl-6-acetylcyclohexene (II).[1,2]

In studying the acetylation of 1-methylcyclohexene with zinc chloride in acetic anhydride, a surprisingly simple result was found. Pure 1-methyl-6-acetylcyclohexene (II) was formed in 70% yield. The purity was indicated by the following observations. Silver nitrate failed to give any precipitate which indicated the absence of chloro compounds. The product remained colorless indefinitely. The index of refraction (n^{20}D 1.4740) was similar to that previously reported for II.[1] The semicarbazone formed in 90% yield and the m.p. (153–155°) was unchanged by recrystallization. In the ultraviolet absorption, the extinction at 248 mμ (ϵ 277) was lower than previously reported for II.[1] This indicated that little if any of the conjugated ketone (I) was present. Actually from our data it can be estimated that the sample of II prepared by Turner and Voitle[1] contained 11% of the conjugated ketone I. This is within the range estimated by these authors.



I II III

Treatment of the pure 1-methyl-6-acetylcyclohexene (II) with sodium methoxide in methanol gave a 95% yield of the equilibrium mixture, which was shown to contain 63% of I and 37% of II.

The reaction conditions used for acetylating methylcyclohexene differed from those of previous workers not only in the use of zinc chloride in place of stannic chloride, but also in the avoidance of prolonged treatment with alkaline reagents. It is entirely possible that II is also the principal product from the stannic chloride catalyzed reaction, but the presence of chloro compounds obscures the exact composition of the initial product.

The exclusive formation of II in the acetylation has an important bearing on the behavior of carbonium ions. The acetylation probably proceeds through the intermediate carbonium ion, III. This carbonium ion must then eject the hydrogen at C-3 rather than the more acidic hydrogen at C-1.

From a consideration of the chlorination of olefins, Taft[3] stated as a general principle (applicable to formation of olefins from carbonium ions) that the most electron-rich carbon, adjacent to the positive carbon, loses the proton. *In the present case* this is equivalent to stating that the least acidic hydrogen is lost. It is evident that the formation of II from the carbonium ion (III) follows this principle.

Experimental

1-Methyl-6-acetylcyclohexene (II).—The procedure was similar to that published for the acetylation of diisobutylene.[4] To a solution of 20 g. of 1-methylcyclohexene in 60 g. of acetic anhydride was added 25 g. of powdered anhydrous zinc chloride. The addition of the solid was completed in 20 min. The temperature rose to 43° during the addition. After stirring for 20 hr., the clear solution was chilled and the excess anhydride decomposed by adding ice and water. Ether (100 ml.) was added and the ether extract washed three times with water, once with dilute potassium hydroxide, and then with water until the washings were neutral. The ether solution was dried over sodium sulfate and distilled to give 20 g. (70%) of colorless 1-methyl-6-acetylcyclohexene (b.p. 77–80° (12 mm.); n^{20}D 1.4740). The product remained colorless on standing.

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