Tetrahedron, 1962, Vol. 18, pp. 477 to 482. Pergamon Press Ltd. Printed in Northern Ireland

KINETICS OF FORMATION AND HYDROLYSIS OF STEROID GIRARD-T HYDRAZONES

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(*Received* 18 *September* 1961)

Abstract—The effect of acetic acid concentration and temperature on the rates of Girard-T hydrazone formation of steroid ketones in methanol have been determined, as well as their effects in the completeness of reaction. The large differences encountered were used to effect selective reactions. Reaction between cholestenone and Girard-T reagent in isopropanol apparently gave a dihydrazide (I). The rates of hydrolysis of the hydrazones at both low and high acidity also showed significant differences.

THE Girard-T reagent (trimethylammonium acetohydrazide chloride, $Me_3\overset{+}{N}CH_2CONHNH_2 \cdot Cl^-$) was first introduced by Girard and Sandulesco[1] for the separation and purification of steroid ketones and has since been repeatedly used for this purpose.[2] They established that the rate of formation of the Girard hydrazones is greatly increased by adding acid, cyclohexanone reacting with Girard-T reagent in a few minutes in methanol containing acetic acid, but taking several hours to react in the absence of acid. They also established from qualitative experiments the order of reactivity of ketones of methylalkyl > alicyclic > methylaryl > diaryl, but no data have been published on the rates of reaction of various steroid ketones. Recently it has been shown that the Girard-T reagent can be titrated iodometrically[3] and this method has now been applied to a study of the reactivity of steroid ketones.

TABLE 1. EFFECT OF ACID ON GIRARD-HYDRAZONE FORMATION[a]

Ketone	M AcOH	$k_f \times 10^2$ l. mole^{-1} sec^{-1}	$K_H \times 10^3$ moles l.$^{-1}$
Cholest-4-en-3-one	—	0·90	[b]
Cholest-4-en-3-one	0·2	2·8	0·33
Cholest-4-en-3-one	1·0	5·4	0·35
Cholest-4-en-3-one	2·5	5·5	2·1
Cholest-4-en-3-one	1·0 (0·2 M NaOAc)	4·6	0·92
Cholest-4-en-3-one	0·2 (at 40°)	4·6	0·88
Cholest-4-en-3-one	1·0 (at 40°)	14·0	[b]
Ergost-8(14)-en-3-one	0·2	25	0·55
Ergost-8(14)-en-3-one	1·0	54	0·94
Estrone methyl ether	0·2	2·0	1·5
Estrone methyl ether	1·0	9·5	2·5

[a] In methanol at 25 ± 1°.
[b] Not determined.

[1] A. Girard and G. Sandulesco, *Helv. Chim. Acta* **19**, 1095 (1936).
[2] O. H. Wheeler, *Chem. Rev.* in press.
[3] O. H. Wheeler, V. S. Gaind and O. Rosado, *J. Org. Chem.* **26**, 3537 (1961).

Cholest-4-en-3-one reacted very slowly in methanol at 25° in the absence of acid (Table I). The rate was greatly increased by adding acetic acid and the reaction was complete (at 0·002 M ketone) with up to 1·0 M acetic acid. Increasing the amount of acid (to 2·5 M) further increased the rate but the reaction was incomplete since the reverse reaction of hydrolysis was catalyzed by strong acid (see below). The rate at 40° (in 0·2 M acetic acid) was only 1·6 times that a 25° corresponding to an energy of activation of 6·2 kcal/mole. The reaction was moreover incomplete at the higher temperature, showing that the overall reaction was exothermic and suggesting that there is little advantage in heating the solution to form the Girard-hydrazone. Increasing the concentration of acid gave similar results with ergost-8(14)-ene-3-one and estrone methyl ether (a 17-ketone). The addition of sodium acetate, in the case of cholestenone, reduced the rate of reaction since it effectively reduced the acidity of the solution.

The rates of reaction of a series of steroid ketones in 0·2 M acetic acid-methanol at 25° (Table 2) established the order $3 > 6 \gg \Delta^4\text{-}3 \sim 7 \sim 17 > 20 > 12 \gg 11$.

TABLE 2. GIRARD-HYDRAZONE FORMATION OF STEROID KETONES[a]

Ketone	$k_f \times 10^3$ l. mole^{-1} sec^{-1}	$K_H \times 10^3$ moles l.$^{-1}$
Cholestan-3-one	43	0·56
Ergost-8(14)-en-3-one	25	0·55
Cholest-4-en-3-one	2·8	0·33
Cholestan-6-one	15	0·62
Cholestan-7-one	2·2	4·4
11-Ketotigogenin	0·015	6·8
Hecogenin[b]	0·47	5·5
Botogenin acetate[b]	0·75	6·6
Androstan-3β-ol-17-one	1·9	1·5
Estrone methyl ether	2·0	1·5
Pregn-4-en-3β-ol-20-one	0·92	2·0
Pregn-5-en-3β-ol-20-one acetate	1·0	1·9

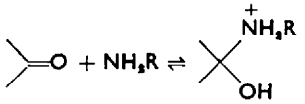
[a] In methanol, 0·2 M acetic acid at 25 ± 1°.
[b] 12-Ketone.

The overall difference in rate was a factor of 2900, with differences of 60 and 30 times between 3-, and 20-, and 3- and 17-ketones. The very slow rate of reaction of 11-ketones explains the fact that these ketones do not react with Girard-T reagent and appear in the "non-ketonic" fraction in Girard separations.[2]

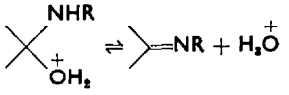
The hydrolysis constants for Girard-T hydrazones (K_H = [reagent][ketone]/[hydrazone]) were calculated (see Table 2) after the solution had reached equilibrium. These constants were relatively small for 3-, Δ^4-3- and 6-ketones (K_H ca. 0·5) indicating that they react nearly completely, moderately large for 17-ketones (K_H ca. 1·5), larger for 20-ketones (K_H ca. 2·0) and much larger for 7-, 12- and 11-ketones (K_H 4–7). These data indicate that a Girard extraction should be complete for 3-, 6- and Δ^4-3-ketones, less complete for 17- and 20-ketones and incomplete for 7-, 12- and 11-ketones. A recent report[4] has stated that Girard-T separations were only complete in the case of 17-ketosteroids (3-ketones were not considered) and that the introduction of other keto groups or double bonds decreased the effectiveness of separation.

[4] L. P. Hansen, *Abstracts, 138th meeting American Chemical Society* p. 57c. New York (1960).

The mechanism of Girard hydrazone formation must involve at least two steps (1 and 2 below) apart from proton-transfer reactions.[5] Both these steps may be rate determining, but the overall reaction showed.



(1)



(2)

second order kinetics and step (1) is probably the slower of the two. In this case the rates of reaction would depend on the steric approach of the reagent to the carbonyl carbon atom (apart from the intrinsic difference between 6- and 5-membered ring ketones). The order of the rates of Girard-T formation was in fact very similar to that found in the rates of reduction with borohydride ion ($3 > 6 > 7 > \Delta^4$-$3 > 12 > 17 > 20 > 11$),[6] a reaction which also involves a change in co-ordination number of 3 to 4. The overall differences in rates of borohydride reduction were however smaller (ratio 3 to 11 ketone 800/1 for borohydride reduction). The extent of Girard hydrazone formation will depend on the relative ease of accommodating the ketone carbonyl group and the much bulkier hydrazine grouping at a certain ring position. The order of hydrolysis constants (Δ^4-$3 \sim 3 < 6 < 17 \sim 20 < 7 \sim 12 \sim 11$) was in accord with this since the hindered 7, 11 and 12 positions showed the lowest degree of hydrazone formation. The order of dissociation constants of cyanohydrins ($7 < 17 < 3 < 6 < \Delta^4$-$3 \ll 20$)[7] was necessarily different since this reaction involves a change in co-ordination number of 4 to 3 and is sensitive to ring angle effects. A brief study of steroid oxime formation[8] gave the order of rates and extents of formation of $3 \gg 4 \sim 6 > 7$ for cholestanones in agreement with that found here.

The large differences between the rates of reaction of 3- and 17-, and 3- and 20-ketones have been used to effect selective reactions at the 3-position. Thus a mixture of cholestan-3-one and estrone methyl ether was treated with one equivalent of Girard-T reagent in 0·2 M acetic acid in methanol, diluted with water and the unreacted estrone methyl ether extracted in 94 per cent yield. Acid hydrolysis of the aqueous solution liberated cholestanone in 90 per cent yield. Allopregnan-3, 20-dione was reacted with one equivalent of Girard-T reagent in acid-methanol solution to form the 3-mono-hydrazone. The acid was neutralized and the 17-keto group reduced with sodium borohydride in methanol and the resulting solution hydrolysed with acid to give allopregnan-20β-ol-3-one. This compound has been previously prepared[9] in 5 steps from allopregnan-3,20-dione. In a kinetic experiment it was shown that the rate constants for hydrazone formation of the 3 and 20 keto groups in the diketone were 37×10^{-2} and $2{\cdot}4 \times 10^{-2}$ mole^{-1} sec^{-1}, respectively.

[5] G. H. Stempel Jr. and G. S. Schaffel, *J. Amer. Chem. Soc.* **66**, 1158 (1944).
[6] O. H. Wheeler and J. L. Mateos, *Canad. J. Chem.* **36**, 1049 (1958); J. L. Mateos, *J. Org. Chem.* **24**, 2034 (1959).
[7a] O. H. Wheeler and J. L. Mateos, *Canad. J. Chem.* **36**, 712 (1958); [b] O. H. Wheeler and O. Rosado, *J. Org. Chem.* **26**, 3016 (1961).
[8] J. Décombe, R. Jaquemain and J. Rabinovitch, *Bull Soc. Chim. Fr.* 447 (1948).
[9] M. Rubin, H. Wishinsky and F. Bompard, *J. Amer. Chem. Soc.* **73**, 2338 (1951).

Experiments were also carried out in isopropanol in which inorganic salts are sufficiently soluble to prepare buffered solutions (Table 3). The rates of reaction were

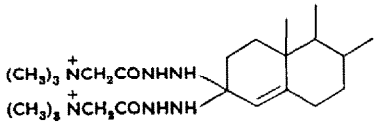
TABLE 3. GIRARD-HYDRAZONE FORMATION IN ISOPROPANOL[a]

Ketone	k_f l. mole^{-1} sec^{-1}	% Reaction
Ergost-8(14)-en-3-one	1·01	140
Cholest-4-en-3-one	0·22	200
Cholestan-6-one	0·30	125
Androstan-3β-ol-17-one	0·24	120
Estrone methyl ether	0·23	110
Allopregn-7-en-3-ol-20-one	0·37	120
Pregn-5-en-3β-ol-20-one acetate	0·43	140

[a] At 25 ± 1°, pH 3·5 (saturated KCl + HCl)

very much faster but the differences very much less than those in methanol. Moreover the second order rate plots deviated from linearity after less than 50 per cent reaction and all ketones reacted with more than one equivalent of Girard-T reagent, up to 2 equivalents in the case of cholest-4-en-3-one. In this case hydrolysis of the solution liberated 100 per cent of the Girard reagent as determined by titration and 90 per cent of the ketone on isolation. The ultraviolet spectra of the Girard-T hydrazone of cholest-4-en-3-one in methanol was normal (λ_{max} 280 mμ, ε 15,700; cf. its semicarbazone λ_{max} 273 mμ, ε 26,300 in ethanol[10]). However the reaction product of this ketone and Girard-T reagent in isopropanol had no maximum above 210 mμ. It is unlikely that addition of the second equivalent of Girard reagent had occurred at the double bond since the Girard-T hydrazones of saturated ketones have maxima at 230–240 mμ,[11] and moreover the saturated steroid ketones also reacted with more than one equivalent.

The product formed with cholest-4-en-3-one most probably had structure I.



Attempts were made to isolate the compound by evaporating the neutralized solution at reduced pressure, extracting the residue with warm methanol and re-evaporating. The oily residue could not be recrystallized. Its infrared spectrum indicated the presence of amine and amide groups and of the C=N grouping. Its ultraviolet spectrum (λ_{max} 277 mμ, ε 6,250) indicated that partial decomposition to the normal hydrazone had occurred.

The selective hydrolysis of Girard-T hydrazones has been used to separate α,β-unsaturated ketones from saturated ones.[2] This is usually carried out by reducing the pH, when the saturated 3-ketones (allopregnane group) are first liberated, the Δ^4-3 ketones (cortisone group) only being hydrolysed at low pH. The rates of hydrolysis of the Girard-T hydrazones were measured by reacting the steroid ketones with an

[10] H. Reich, F. E. Walker and R. W. Collins, *J. Org. Chem.* **16**, 1753 (1951).
[11] J. R. Young, *J. Chem. Soc.* 1516 (1955).

excess of Girard reagent in 0·2 M acetic acid in methanol for 24 hours and then lowering the pH to 1·5. The first order rate plots were then calculated from the amount of Girard-T reagent liberated in the hydrolysis (Table 4). The corresponding

TABLE 4. KINETICS OF HYDROLYSIS OF GIRARD-HYDRAZONES[a]

	pH 1·5		pH 3·5[b]	
	$k_h \times 10^5$	$K_H \times 10^3$	$k_h \times 10^5$	$K_H \times 10^3$
Cholestan-3-one	127	7·2	24·0	0·56
Cholest-4-en-3-one	25·6	1·1	9·25	0·33
Cholestan-6-one	—	—	9·3	0·62
Cholestan-7-one	—	—	9·7	4·4
Androstan-3β-ol-17-one	21·5	4·0	2·85	1·5
Estrone methyl ether	23·8	3·1	2·9	1·5
Pregn-5-en-3β-ol-20-one	15·4	—	2·0	1·9

[a] In methanol at 25 ± 1°.
[b] In 0·2 M acetic acid, k_h calculated from hydrolysis constants and rates of formation.

rates of hydrolysis (k_h) at pH 3·5 (0·2 M acetic acid) were also calculated from the known hydrolysis constants (K_H) and rates of formation (k_f) ($k_h = K_H \cdot k_f$). At pH 1·5 the 3 ketosteroid was hydrolysed at a greater rate (ca. 3X) than the Δ^4-3-, 17- and 20-ketones which all hydrolysed at similar rates. However at pH 3·5 the rates, which were lower by a factor of 3–8, fell into three groups, the 3-ketone, then the Δ^4-3-, 6- and 7-ketones and lastly the 17- and 20-ketones, the differences being about 10:4:1. The hydrolysis constants (K_H) at pH 1·5 were lower than at pH 3·5, the order being 3 > 17 > Δ^4-3 ketone and the 3-ketone hydrolysing to a relatively greater extent than at higher pH. The observation that 3-ketones hydrazone are liberated first at higher pH than those of Δ^4-3-ketones must be due to their higher dissociation constants as well to their relatively faster rates of hydrolysis. The smaller differences found in the rates of hydrolysis and the hydrolysis constants, as compared to the rates of formation, suggest that selective formation of Girard hydrazones would be more efficient for achieving separations of mixture of steroid ketones.

EXPERIMENTAL

Reagents. Cholestan-3-one, cholestan-6-one, cholestan-7-one, cholest-4-en-3-one, and ergost-8(14)-en-3-one were prepared by standard methods; the other ketones were analytically pure samples. Girard-T reagent was recrystallized from ethanol and stored in a desiccator.

Kinetics in methanol. The ketone (ca. 80 mg) was dissolved in 90% methanol containing the required amount of acetic acid, allowed to equilibrate in a const. temp. bath at 25 ± 1° and 10 ml of a 0·05 M Girard-T solution in methanol added. Aliquots of 10 ml were withdrawn at intervals, added to 10–25 ml aqueous phosphate buffer (pH 7) containing 5 ml 0·01 N iodine; and the excess iodine titrated with 0·05 N thiosulfate using starch as indicator. The rate constants were calculated from the slopes of second order rate plots. The reactions were titrated after 24 hr to determine the equilibrium constants.

The kinetics of reaction were similarly determined in isopropanol containing 0·5 M potassium chloride to which had been added a few drops of conc hydrochloric acid to give a pH of 3·5.

The spectra were determined by first titrating an aliquot to determine the amount of Girard-T reagent which had reacted, and then diluting another aliquot with either methanol or isopropanol to give an optical density <0·8.

The kinetics of hydrolysis were carried out by reacting the ketone in 0·2 M acetic acid–methanol for at least 24 hr at 25°, adding a few drops of conc hydrochloric acid to give a pH of 1·5 and titrating aliquots at intervals. The amount of hydrozone hydrolysed was calculated from the amount of Girard-T reagent reacted before acidification (i.e. the amount of hydrazone formed) and the amount of Girard-T reagent present in solution at each interval. The data were treated as first-order rate plots.

The determinations were carried out in duplicate or triplicate and the mean values with errors of ±5% are given in Tables 1–4.

In isopropanol. Cholest-4-en-3-one (0·25 g) in isopropanol (50 ml), pH 3·5 (saturated potassium chloride plus few drops of saturated hydrochloric acid) was reacted with Girard-T reagent (0·25 g) for 24 hr, neutralized with excess solid potassium carbonate and evaporated at red press at <35°. The pasty residue was extracted with warm methanol which was evaporated again at red press at <25°. The oily residue could not be recrystallized from methanol, benzene and chloroform.

Infrared spectrum in carbon tetrachloride: 3500 (strong) (NH unbonded), 3050 (weak) (NH bonded), 1690 (strong) (amide I), 1650 (strong) C=N) and 1570 cm^{-1} (medium) (amide II).[12] Ultraviolet spectrum in methanol: λ_{max} 277 mμ, ε 6,250.

Separation experiments. Cholestan-3-one (390 mg) and estrone methyl ether (280 mg) in 0·2 M acetic acid in methanol (200 ml) were reacted with Girard-T hydrochloride (170 mg; 1 equivalent) for 24 hr at room temp. The solution was diluted with water and extracted with ether. Evaporation of the ether extract gave estrone methyl ether (260 mg), m.p. 168°. The aqueous solution was acidified with dil hydrochloric acid, allowed to stand for 6 hr and then extracted with ether. Evaporation of the ether gave cholestanone (350 mg), m.p. 128° from ethanol.

Allopregnan-3,20-dione (0·22 g) in 0·2 M acetic acid methanol (100 ml) was reacted with Girard-T reagent (0·12 g, 1 equivalent) for 24 hr 5% sodium hydroxide was added to pH 8 and then sodium borohydride (0·026 g; 1 equivalent). After 24 hr the solution was extracted with ether (evaporation of the extract gave only a trace of residue), acidified with dil hydrochloric acid and again extracted with ether giving a residue (0·20 g), m.p. 189–190°, $[\alpha]^{24}$ +20·0°. Reported for allopregnan-20-β-ol-3-one m.p. 187–189°[9], 195°[13] and $[\alpha]^{24}$ +18·5°[9].

Acknowledgements—The authors are grateful to Syntex, SA., Mexico for financial assistance and for gifts of steroids.

[12] W. H. T. Davison and P. E. Christie, *J. Chem. Soc.* 3389 (1955).
[13] R. E. Marker, O. Kamm, D. M. Jones and T. S. Oakwood, *J. Amer. Chem. Soc.* **59**, 614 (1937).