[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE UNIVERSITY]

LEAD TETRAACETATE OXIDATION OF STEROIDAL GLYCOLS. SOME OBSERVATIONS ON OUABAGENIN

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Received February 22, 1954

Although the cardiac glycoside ouabain has been known since 1888 (1), structural studies on the intact aglycone, ouabagenin, have only been possible since 1942 when Mannich and Siewert (2) described a hydrolysis procedure (acetone and hydrochloric acid) which did not cause alterations in the aglycone molecule. Of the eight oxygen atoms in ouabagenin ($C_{23}H_{34}O_8$), two are found in the conventional butenolide side chain (1) and the remaining six are present as hydroxyl functions of which four can be acetylated, the two non-reactive ones presumably being attached as tertiary hydroxyl groups at C-5 and C-14. Ouabagenin is thus the most highly oxygenated cardiac aglycone and in spite of several investigations (1–4)[1] no unequivocal structure has as yet been presented.

In their original hydrolysis studies of ouabain, Mannich and Siewert (2) first isolated ouabagenin acetonide which upon further acid treatment yielded ouabagenin. The aglycone, however, could not be reconverted to the acetonide and did not react with lead tetraacetate; on the basis of these observations, the presence of a 1,3-glycol grouping was postulated. In view of the fact that certain steroidal *trans*-1,2-glycols [*e.g.* gitogenin (I)] of established constitution do not form acetonides (5) and react only very slowly with lead tetraacetate,[2] it was felt that a more detailed study of such oxidations in the steroid series together with a re-investigation of "ouabagenin acetonide" might shed some light on the structure of this aglycone.

The substrates chosen for the present lead tetraacetate oxidations were steroidal sapogenins, since a number of them contain 1,2-glycol groupings (6). 22a, 5α-Spirostane-2α, 3β-diol (gitogenin) (I) may be considered the parent substance of this class since several other glycol-containing sapogenins (lilagenin, yuccagenin, kammogenin, mannogenin) have been correlated with it (6). It has recently been established (5, 7) that gitogenin (I), and hence the above mentioned sapogenins, contain the di-equatorial *trans* 2α,3β-diol grouping and this sapogenin was, therefore, considered a suitable reference compound in our oxidation studies, all of which were carried out at 25° in glacial acetic acid. For the sake of convenience, all curves were converted into rate constants ($k \times 10^3$ lit-mole^{-1} sec.$^{-1}$) by the procedure of Cordner and Pausaker (8) and the results are summarized in Table I.

Using the di-equatorial 2α,3β-*trans* glycol gitogenin (I) as the standard, it can be seen that both possible *cis* glycols, 2α,3α (II) and 2β,3β (IV)[3] are oxidized at a

[1] The work of Sneeden and Turner appeared after our experiments described in this paper had already been completed.

[2] Previously unpublished observation.

[3] We are grateful to Drs. J. Herran and F. Sondheimer of the National University of Mexico for a gift of this substance, the synthesis of which will be described elsewhere by them.

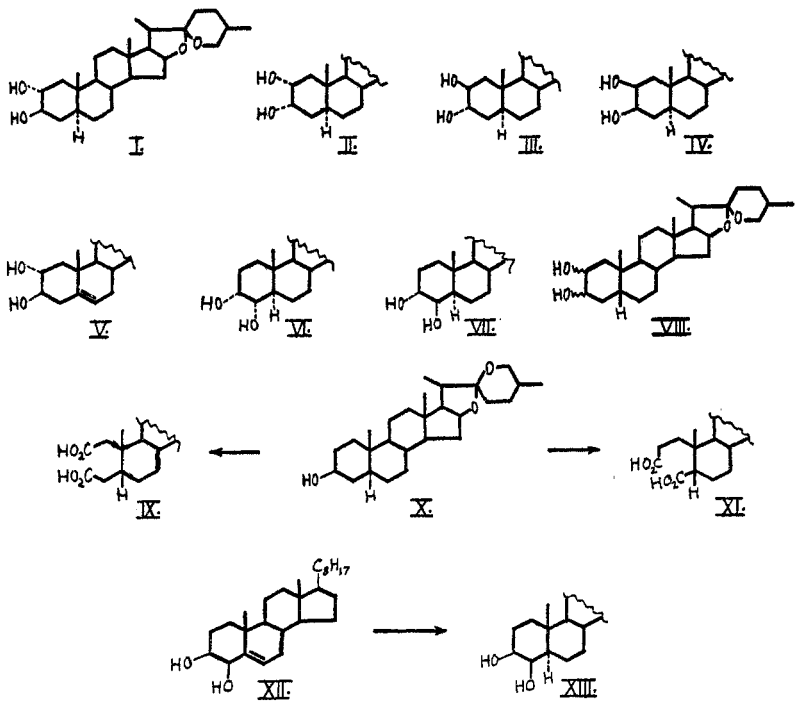
TABLE I

LEAD TETRAACETATE OXIDATION OF STEROIDAL GLYCOLS

Compound	$k \times 10^3$
Gitogenin (I) (22a,5α-Spirostane-2α,3β-diol)	1.85
22a,5α-Spirostane-2α,3α-diol (II)	13.2
22a,5α-Spirostane-2β,3α-diol (III)	5.02
22a,5α-Spirostane-2β,3β-diol (IV)	3.69
22a,5α-Spirostane-3α,4α-diol (VI)	112.6
22a,5α-Spirostane-3α,4β-diol (VII)	0.0357
Cholestane-3β,4β-diol (XIII)	91.3
Samogenin	141.6
Markogenin	157.5
Yuccagenin (V) (Δ⁵-22a-spirostene-2α,3β-diol)	6.33
Kammogenin (V with 12-keto group) (Δ⁵-22a-spirostene-2α,3β-diol-12-one)	6.31
Δ⁵-Cholestene-3β,4β-diol	494.6
Ouabagenin	0.0834

faster rate, an observation which is in agreement with the fundamental studies of Criegee and co-workers (9) on the oxidation of the *cis* and *trans* cyclohexane- and cyclopentane-1,2-diols. Of interest is the observation that the isomeric di-axial *trans*-glycol, 22a, 5α-spirostane-2β,3α-diol (III) was oxidized nearly three times as fast as the di-equatorial gitogenin (I) and even faster than one of the *cis* glycols (IV). A further illustration of the subtle effects upon rate of oxidation in this steroidal glycol series can be found in the behavior of yuccagenin (V) and kammogenin (V with keto group at C-12). These sapogenins differ from our standard gitogenin (I) only in possessing a 5,6-double bond but are otherwise stereochemically identical. Nevertheless, yuccagenin (V) and kammogenin were oxidized 3.5 times faster than gitogenin (I). That the oxidation of the 5,6-double bond *per se* is not involved is shown by the fact that the consumption of the oxidizing agent does not proceed beyond one equivalent and even more clearly by the stability of diosgenin (Δ⁵-22a-spirosten-3β-ol) towards lead tetraacetate. Since the rate-determining step in lead tetraacetate oxidations is believed (8, 9) to involve the O—H rather than C—H bond, it is conceivable that the activating influence of the 5,6-double bond upon the rate of 2,3-glycol splitting may involve intermediates invoked in i-steroid reactions.

Since we had now available a series of characteristic rates on the lead tetraacetate oxidation of several steroidal sapogenin 2,3-glycols, it appeared of interest to examine the behavior of samogenin and markogenin under these conditions. Samogenin was originally isolated by Marker, *et al.* (10) who assigned to it the structure 22a-spirostane-2,3-diol (VIII) on what we consider to be ambiguous grounds. The presence of the glycol grouping was demonstrated by chromium trioxide oxidation to a dibasic acid, different from the corresponding oxidation product of gitogenin (I) and this fact, together with the observation that samogenin could be epimerized with sodium ethoxide to "episamogenin" and formed a rather soluble digitonin complex, was presented as evidence in



favor of the 5β (normal) configuration. Subsequently, it was reported (11) that "neosamogenin" (22b-spirostane-2,3-diol) on chromium trioxide oxidation yielded a dibasic acid (IX) identical with that obtained from sarsasapogenin (X) (12). In spite of Marker's statements (*cf.* 13), it appears to us that this acid could also have the 3,4-seco structure (XI) which is observed quite frequently in the oxidation of steroids with the 5β-configuration. Some inconsistencies in Marker's work have come to light in the report of Wall, *et al.*, (14) who have recently isolated a dihydroxy sapogenin, named markogenin which can be isomerized with ethanolic hydrochloric acid to samogenin and which should, therefore, be identical with Marker's "neosamogenin" (X with additional hydroxyl group at C-2).[4] The physical constants for markogenic acid recorded by Wall and co-workers (14) differ completely from those reported (12) for sarsasapogenic acid and a similar disagreement obtains in the case of markogenin and neosamogenin. In conclusion it appears that while certain of the above cited observations are mutually inconsistent, the evidence at the present time does not exclude a 3,4-dihydroxy glycol structure for samogenin and markogenin. Quite pertinent to this discussion, therefore, is a consideration of the results obtained in the lead tetraacetate oxidation. Both substances are oxidized at essentially the same rate

[4] On the other hand, markogenin gives a pseudo derivative different from that of samogenin which would indicate that these compounds are possibly C-25 epimers [Scheer, Kostic and Mosettig, *J. Am. Chem. Soc.*, **75**, 4871 (1953)] or epimeric at both C-22 and C-25 [Wall and Serota, *J. Am. Chem. Soc.*, **76**, 2850 (1954)].

(Table I), which would tend to support the supposition that rings A and B in both sapogenins are identical, but the remarkable fact is that the rate of oxidation is nearly eighty times faster than that observed for gitogenin (I).

It is as yet impossible to assess the effect of a 5β ring juncture since authentic steroidal sapogenin glycols with the 5β-configuration are unknown,[5] but it has been possible to examine the behavior of some 3,4-dihydroxy steroids of the 5α-configuration. Two of these, the 3α,4α-diol (VI) and 3α,4β-diol (VII) were available in the sapogenin series (15), while a 3β,4β-diol (XIII) has been described (16) in the cholesterol series where it has been prepared by hydrogenation of Δ^5-cholestene-3β,4β-diol (XII). The very striking oxidation results with these four compounds are listed in Table I and particularly noteworthy is the exceedingly rapid oxidation of the *cis* 3α,4α-diol (VI) and the extremely slow oxidation (450 hrs.) of the di-axial *trans* 3α,4β-diol (VII). The slightly slower rate of oxidation of cholestane-3β,4β-diol (XIII) as compared to the α-diol VI agrees with expectation while the activating influence of the 5,6-double bond (XII)—now by virtue of allylic activation—manifests itself in the expected direction and indeed results in the fastest rate of oxidation observed so far (5 min.). With these results at hand, it can be seen that the rate of oxidation of samogenin and markogenin most closely resembles that of 22a, 5α-spirostane-3α,4α-diol (VI) and while these compounds are not identical, the fast rate of oxidation makes a 3,4-diol structure (*e.g.* 3β,4α) equally plausible. An unequivocal decision can be reached only when certain model diols with the 5β-configuration become available.[5]

The above results indicate clearly that comparatively minor structural changes may have an enormous effect on the rate of glycol splitting with lead tetraacetate. With this observation in mind, we have re-investigated the lead tetraacetate oxidation of ouabagenin, since Mannich and Siewert (2) did not indicate the time or temperature at which the negative lead tetraacetate oxidation was observed. In our hands, ouabagenin consumed nearly one equivalent of lead tetraacetate in 190 hours and 1.6 equivalents in 196 additional hours. It is questionable that the reagent may have reacted with other groups (*e.g.* the lactone grouping) during this long period of time since β-ouabagenin diacetate or ouabagenin acetonide (see below) consumed only 0.15 equivalent of the reagent in 100 hours. Sneeden and Turner (4) arrived at the absence of a *vic*-glycol grouping in ouabagenin by a short time (30 minutes) periodic acid titration. From our results it would appear that ouabagenin possesses a *vic*-diol and conceivably even a triol system.

The key compound — ouabagenin acetonide — had been reported (2, 3) as a very insoluble substance (m.p. 200° and 255°) which could not be recrystallized and which upon boiling with nitrobenzene was assumed to be converted into "anhydro-ouabagenin" (m.p. 305°) supposedly involving loss of the acetone grouping and oxide formation of the liberated 1,3-glycol. The stereochemical impossibility of such a formulation has already been pointed out by Sneeden

[5] The synthesis of such diols is contemplated in this laboratory in the hope that this will clarify the structure assignments of samogenin and markogenin.

and Turner (4) who have shown that these two substances are, in fact, identical. We have arrived at the same conclusion independently and are reporting briefly our results where they extend the work of these investigators. We found that the acetonide could be crystallized quite readily from dimethyl formamide and that the resulting pure substance could be cleaved with acid to yield ouabagenin and acetone (74% yield as the DNPH derivative). The pure acetonide could *not* be cleaved by heating with ethanol as has been reported earlier (2, 3) for the crude product and this was now shown to be due to the presence of small amounts of acid in the crude product. The unusual observation (2) that ouabagenin cannot be reconverted into its acetonide in spite of the fact that this acetonide was originally formed during the acetone-hydrochloric acid hydrolysis of ouabain has now been found to be incorrect. Treatment of ouabagenin with acetone-hydrochloric acid, precisely the conditions employed for the hydrolysis of ouabain, resulted in the expected formation of ouabagenin acetonide as shown by infrared comparison. It is clear, therefore, that in this respect at least, ouabagenin behaves like a normal 1,2- or 1,3-glycol. Using the pure acetonide, we have also confirmed the formation of an acetonide diacetate and its cleavage to acetone and ouabagenin α- or β-diacetate depending upon the acid employed. The formation of two isomers has been ascribed (3) to acyl migration.

Acknowledgment. We are grateful to Dr. W. G. Bywater of S. B. Penick & Co. for a generous gift of ouabain and to Syntex, S. A., Mexico City and Dr. M. E. Wall of the Eastern Regional Research Laboratory for sapogenin specimens.

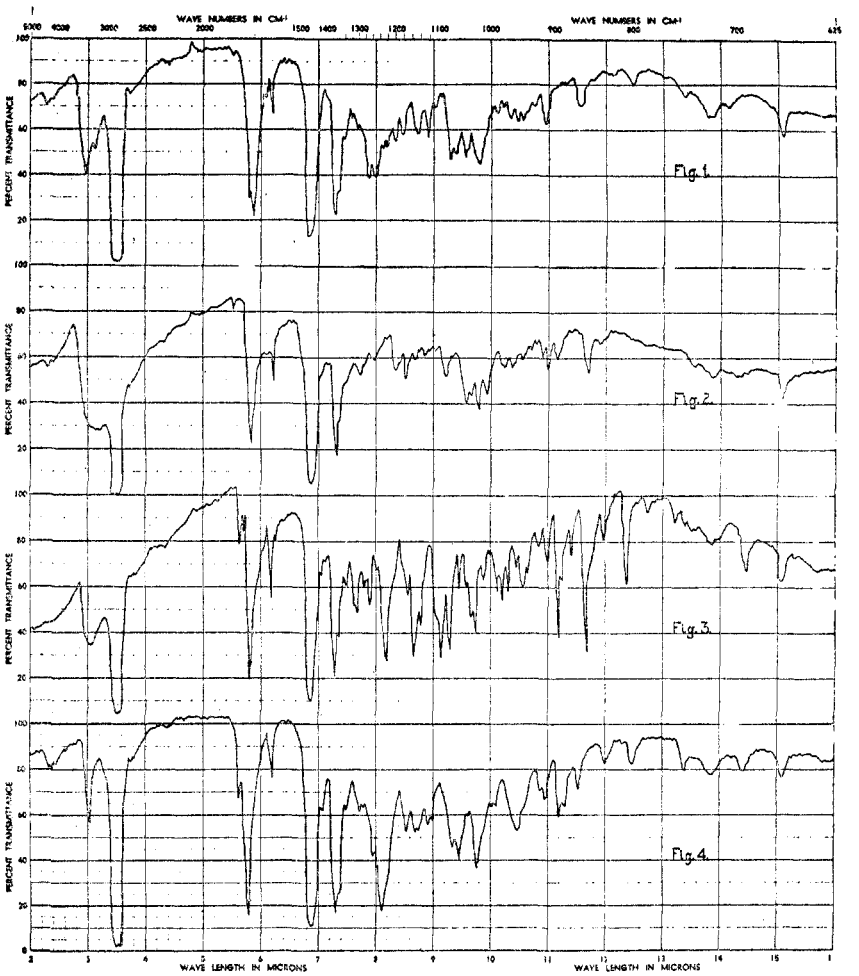
EXPERIMENTAL[6]

Ouabagenin acetonide. (a) By hydrolysis of ouabain. A suspension of 9.7 g. of ouabain (U.S.P. grade, S. B. Penick) in 500 cc. of acetone was treated with 5.5 cc. of conc'd hydrochloric acid and was shaken for 20 minutes. A small amount of insoluble material (0.38 g., m.p. > 300°) was filtered, and the solution was seeded with a crystal of acetonide and permitted to stand at room temperature for two weeks. Filtration afforded 4.57 g. of crystals with m.p. 193–211°, the m.p. of the crude material ranging in different runs from 175–250°. Further standing of the filtrate for 7 days resulted in the deposition of an additional 0.22 g. of acetonide with m.p. 231–239° which proved to be identical (infrared comparison) with the material from the first crop.

A sample (4.6 g.) of the crude acetonide was ground to a fine powder and was digested for 3 hours with 15 cc. of dimethylformamide on the steam-bath and cooled. Collection of the crystals, washing with acetone,[7] and drying furnished 3.54 g. of colorless crystals with m.p. 302–304° (dec.), infrared spectrum in Fig. 3. Approximately 0.15 equivalent of lead

[6] Melting points were determined in a Dowtherm bath and are uncorrected. Rotations were measured in dioxane solution, while the infrared spectra were obtained with a Baird double beam infrared spectrophotometer in Nujol mulls. The microanalyses were performed by Mr. Joseph F. Alicino, Metuchen, New Jersey.

[7] In order to demonstrate that the acetone isolated in the subsequent acid treatment arose from the cleavage of the acetonide grouping rather than from solvent of crystallization, a small sample of the acetonide was dissolved completely in boiling dimethylformamide and after cooling, the crystals were filtered and allowed to suck dry. Cleavage of 30 mg. of such material gave 50% of acetone 2,4-dinitrophenylhydrazone.



INFRARED SPECTRA IN NUJOL MULLS

FIG. 1. β-Ouabagenin diacetate
FIG. 2. Ouabagenin
FIG. 3. Ouabagenin acetonide
FIG. 4. Ouabagenin acetonide diacetate

tetraacetate was consumed in 100 hrs. which was probably due to partial cleavage of the acetonide grouping in acetic acid.

Anal. Calc'd for $C_{26}H_{38}O_8$: C, 65.25; H, 8.00.
Found: C, 65.24; H, 7.79.

A sample of acetonide (0.24 g., m.p. 290°) was refluxed with 13 cc. of 50% ethanol solution for 3 hours without effecting solution or liberation of acetone. Addition of one drop of 5% hydrochloric acid caused immediate solution and distillation into Brady's solution yielded 0.089 g. (74%) of acetone 2,4-dinitrophenylhydrazone with m.p. 124–125°.

In view of the report (2) that the crude acetonide could be cleaved by boiling with ethanol alone, 0.24 g. of crude acetonide (m.p. 210–218°) was refluxed with 13 cc. of 50% ethanol for 20 minutes resulting in complete solution, in marked contrast to the behavior

of the pure acetonide. Distillation of the ethanol into Brady's solution yielded 0.076 g. (65%) of acetone 2,4-dinitrophenylhydrazone. However, when this experiment was repeated except that 0.055 g. of sodium carbonate was added, no acetone was liberated after 2 hours refluxing. Evaporation of the solution to dryness yielded acetonide with m.p. 280–283°. Cleavage of this material in ethanol containing one drop of 5% hydrochloric acid immediately furnished 60% of acetone 2,4-dinitrophenylhydrazone.

(*b*) *From ouabagenin.* A solution of 0.107 g. of ouabagenin (m.p. 221–225°) in 5 cc. of acetone and 0.5 cc. of conc'd hydrochloric acid was allowed to stand at room temperature for 3 weeks. Collection of the precipitate furnished 0.094 g. of acetonide with m.p. 250–262°, raised on treatment with dimethylformamide to 285–288° (dec.); identity with acetonide according to (*a*) was established by mixture melting point determination and infrared comparison.

(*c*) *Identity with "anhydro-ouabagenin."* The crude acetonide [0.24 g., m.p. 178–196° (dec.)], was refluxed with 4 cc. of nitrobenzene for 5 minutes in order to effect solution, cooled, ethanol was added, and the solution was concentrated by distillation into Brady's solution; no 2,4-dinitrophenylhydrazone was formed. Chilling and filtration of the crystals in the distillation flask furnished 0.07 g. of acetonide (infrared comparison) with m.p. 280–283°. When this material was suspended in 4 cc. of 50% ethanol containing 1 drop of 5% hydrochloric acid and was distilled into Brady's solution, 73% of acetone 2,4-dinitrophenylhydrazone was isolated. These observations establish conclusively the identity of Mannich and Siewerth's "anhydroöuabagenin" (2) with ouabagenin acetonide; the supposed difference has already been questioned earlier by Raffauf and Reichstein (3).

Cleavage of ouabagenin acetonide to ouabagenin. A suspension of 2.14 g. of recrystallized acetonide in 25 cc. of 50% ethanol and 5 cc. of acetic acid was refluxed for 30 min. whereupon a clear solution resulted. After 3 hrs. at room temperature, the volume was reduced and the precipitate was collected and dried; yield, 1.33 g., m.p. 221–225° (dec.). Trituration of a sample with hot 1-butanol raised the m.p. to 237–239° (dec.) while recrystallization by dissolving in a large volume of cold 95% ethanol and concentration furnished crystals with m.p. 242–244° (dec.), $[\alpha]_D^{20}$ −48°. The infrared spectra of all three samples were identical and that of the m.p. 242–244° material is depicted in Fig. 2. Lead tetraacetate oxidation of such a sample required 190 hrs. for the consumption of one equivalent of reagent and an additional 196 hrs. for a further 0.6 equivalent.

Ouabagenin acetonide diacetate. The pure acetonide (3.37 g.) was dissolved in 135 cc. of pyridine and 14 cc. of acetic anhydride and left at room temperature for two weeks. Evaporation to dryness *in vacuo*, solution in chloroform, washing in the standard manner with dilute acid and sodium carbonate, followed by drying and evaporating yielded the diacetate which after several recrystallizations from ethyl acetate was obtained as colorless crystals (2.46 g.) with m.p. 272–274° (dec.), $[\alpha]_D^{24}$ +72°; infrared spectrum Fig. 4.

Anal. Calc'd for $C_{30}H_{42}O_{10}$: C, 64.04; H, 7.52; Acetyl, 15.30.
Found: C, 64.00; H, 7.73; Acetyl, 15.23.

Hydrolysis of a sample of this acetonide in the usual manner with ethanolic hydrochloric acid resulted in the isolation of 94% of acetone 2,4-dinitrophenylhydrazone. From a preparative standpoint, the hydrolysis was best effected with dilute acetic acid according to the method of Sneeden and Turner (4) since this yielded only β-ouabagenin diacetate, m.p. 192–195° (dec.) $[\alpha]_D^{24}$ −9.2°, infrared spectrum in Fig. 1. An inspection of the hydroxyl region of the various infrared spectra is instructive. Ouabagenin and (to a much smaller extent) the acetonide show hydrogen bonding without any resolution in the 3 μ region, while the acetonide diacetate (Fig. 4) shows only a sharp band at 3.02 μ and β-ouabagenin diacetate (Fig. 1) exhibits two well-defined bands at 3.0 and 3.1 μ. Another noteworthy difference is the somewhat unusual shape of the bond (8 μ region) associated with the acetate C—O stretching vibration in β-ouabagenin diacetate (Fig. 1). The position of the carbonyl bands of the various ouabagenin derivatives is in agreement with that observed for other cardiac aglycones (*cf.* 17).

Lead tetraacetate oxidations. The procedure was essentially that of Hockett, *et al.* (18),

employing lead tetraacetate from Arapahoe Chemicals, Boulder, Colo., and Baker's 99.8% glacial acetic acid. The lead tetraacetate was made up approximately 0.1 *N* by dissolving 14 g. in 500 cc. of acetic acid. The sample (0.25 mmol.) dissolved in 20–30 cc. of acetic acid was mixed with 15.2 times the molar excess of lead tetraacetate and the volume was adjusted to 100 cc. with glacial acetic acid. Periodically, 10-cc. samples were withdrawn, and added to 25 cc. of an aqueous solution of 0.5 g. of sodium iodide and 5 g. of sodium acetate, the liberated iodine being titrated with 0.02 *N* sodium thiosulfate solution. All oxidations were run at 25° and appropriate blanks were run with each determination.

The rate constants for the oxidations were calculated as described by Cordner and Pausacker (8). The $\log \left[\frac{a(b-x)}{b(a-x)}\right]$ was plotted against time t; (a = initial concentration of diol, b = initial concentration of lead tetraacetate, x = amount reacted at time t). The slope of the resultant straight line was calculated and substituted in the equation

$$k = \frac{2.303 \times \text{slope}}{(b-a)} = \text{lit-mole}^{-1}\text{-sec}^{-1}$$

SUMMARY

Quantitative data on the rate of lead tetraacetate oxidation of epimeric steroidal 2,3- and 3,4-glycols are reported and compared with those of the steroidal sapogenins markogenin and samogenin and the cardiac aglycone ouabagenin. The latter appears to possess a *vic*-glycol system.

DETROIT 1, MICHIGAN

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