



Le spectre de masse montre un pic moléculaire à  $m/e$  404 correspondant à la formule  $C_{24}H_{20}O_6$ . Les autres pics importants sont à  $M^+ - 42$  ( $m/e$  362) et  $M^+ - 42 - 60$  ( $m/e$  302). Par analogie avec le cas du 2,3,6,8,9-pentaacétoxy-12-(acétoxy-méthyl)-benzo-[1,2-b;4,5-b']-bisbenzofurane<sup>3</sup> la fragmentation préférée semble être la coupure du groupement acétoxy aromatique ( $CH_2CO$ ) suivie par la perte de l'acétoxy aliphatique sous forme de  $CH_3COOH$ . L'existence de forts pics métastables à  $m/e$  324,5 et 252 semble confirmer cette séquence de coupure mais il existe aussi un pic à  $m/e$  319 correspondant à  $M^+ - 42 - 43$ .

Le spectre RMN montre un singulet de deux protons à  $\tau$  4,07 ( $-O-CH_2-O-$ ), un singulet de deux protons à  $\tau$  5,10 ( $-CH_2-O-CO-$ ) et, dans la zone aromatique, dix protons centrés à  $\tau$  2,58. En plus, il y a un singulet de six protons à  $\tau$  8,03 ( $-O-CO-CH_3$ ). Le déplacement chimique des protons benzyliques ( $-CH_2-O-CO-$ ) du 1-acétoxy-2-(acétoxy-méthyl)-3,6-diphényl-4,5-méthylène-dioxy-benzène (6) ( $\tau$  5,10) est très différent de celui du 2,3,6,8,9-pentaacétoxy-12-(acétoxy-méthyl)-benzo-[1,2-b;4,5-b']-bisbenzofurane (5) ( $\tau$  4,13).<sup>3</sup> Dans celui-ci tous les noyaux sont dans le même plan; dans celui-là les noyaux aromatiques latéraux sont perpendiculaires, ou presque, par rapport au plan du noyau central, donc les substituants sont situés au-dessus de ces noyaux latéraux et, par conséquent, les protons résonnent à une valeur du champ plus élevée.

La structure proposée est confirmée par le spectre IR, qui montre deux maxima dans la région de  $\nu C=O$  ( $1774\text{ cm}^{-1}$  et  $1746\text{ cm}^{-1}$ ) dont le premier est celui de l'acétate aromatique.

Le spectre IR a été effectué (produit en pastille de KBr) sur spectrographe Unicam SP 100. Le spectre RMN a été réalisé dans le deutérochloroforme avec l'appareil Varian A 60. Le spectre de masse a été exécuté sur spectrographe A.E.I. MS 9.

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## Halogenation of Ketones

### VI.\* On the Deuteration of Butanone-2 in Aqueous Acetate, and Acetate Buffers

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The enolization of keto-compounds has been carefully studied during the last years.<sup>1-3</sup> In most of these investigations the enolization was studied by halogenation reactions. For recent works in this field see Refs. 4-7. However, very recently the present author reported experiments, which indicated that enols do not take part in the halogenation reactions.<sup>8</sup>

In a limited number of publications the enolization of keto-compounds has been studied by deuteration experiments. Hine *et al.* have studied the deuterium exchange of isobutyraldehyde-2-*d* in aqueous buffers of tertiary amines and oxygen bases.<sup>9</sup> In these buffers they observed large deviations from the Brønsted catalysis equation,

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especially for 2- and 6-substituted pyridines. These deviations were explained on the basis of steric hindrance.<sup>9</sup> In other papers the stereochemistry of the deuteration of some 17-keto steroids and bicyclo-[2,2,1]heptanones has been studied.<sup>10-12</sup>

In connection with studies on the base catalyzed halogenation of ketones and the mechanism of these reactions<sup>8,13</sup> the present author recently investigated the deuteration of butanone-2.<sup>14</sup> Here the deuteration of the two  $\alpha$ -carbons was studied and a ratio  $K_D = 3\text{-deuteration}/1\text{-deuteration}$  was determined. In acid catalyzed reactions a  $K_D$ -value of 2.4–2.6 was found, and in base catalyzed reactions the  $K_D$ -value was estimated as 0.65–0.70. Among the bases were sodium acetate and sodium deuterioxide. They both gave the same  $K_D$ -values. The deuteration was also studied in pure  $D_2O$  ( $K_D = 1.6$ ) and in acetate buffers ( $K_D = 0.8\text{--}1.7$ ).<sup>14</sup>

It can be noted that the catalytic rate constants for the deuteration of butanone-2 given in Ref. 14 using the catalysts  $D_3O^+$ ,  $D_2O$ ,  $OD^-$  and acetate correspond well with the values given by Hine *et al.*<sup>9</sup> for the deuterium exchange of isobutyraldehyde-2-*d*.

Shortly after Ref. 14 was sent for publication, a paper by Warkentin and Tee appeared.<sup>15</sup> These authors have also studied the base catalyzed deuteration of butanone-2, using as bases sodium deuterioxide, sodium *p*-nitrophenoxide and sodium acetate. Contrary to the result in Ref. 14 they found differing  $K_D$ -values for different bases. For sodium deuterioxide they reported the value  $K_D = 0.65\text{--}0.70$ , which is the same as that reported in Ref. 14, but for sodium acetate a  $K_D$ -value of 1.44 was given (Ref. 14 gives  $K_D = 0.65\text{--}0.70$  for deuterations with this base). From their data Warkentin and Tee state that the enolization in dilute hydroxide does *not* go by the currently accepted carbanion mechanism.<sup>15</sup>

The primary interest in Ref. 14 was to study the deuteration of butanone-2 and to compare this with the halogenation under similar conditions. Therefore, the  $K_D$ -values were in all cases calculated from the observed (and total) rate constants. However, the observed (and total) rate constants are made up by the following components:

$$k_{\text{obs}} = v_0 + k_{OH^-} [OH^-] + k_B [B] + k_{HB} [HB] + k_{H_3O^+} [H_3O^+] \quad (1)$$

where  $v_0$  is due to catalysis by water molecules.

The contribution from the undissociated acid,  $k_{HB} [HB]$ , was studied by Hine *et al.*<sup>9</sup> Since they obtained the same value for the catalytic constant of acetate ions with a buffer ratio 1:1 as with a buffer ratio 7.2:1, they concluded that catalysis by undissociated acetic acid does not contribute significantly to the reaction.<sup>9</sup> It can be objected that this method may be rather cursory. The observed rates can be the sum of the rates of two reactions, one acid catalyzed and one base catalyzed. The sum of the rates of these two reactions can be the same even if the rates of the separate reactions differ.

In the deuteration of butanone-2, where acid and base catalysis gave different  $K_D$ -values, the influence of the undissociated acid can be calculated from the  $K_D$ -values and the catalytic rate constants in Ref. 14 and the observed rate constants. In Table 1 the rate constants for different values of the buffer ratio are given together with the amount of  $CH_2$ -deuteration taking place *via* an acid catalyzed reaction. In the case of butanone-2 a buffer ratio 1:1 (expt. 1) gave almost exclusively base catalyzed deuteration, while in a buffer with the ratio 5.4:1 (expt. 2) about 20 % of the deuteration occurred *via* an acid catalyzed reaction.

From halogenation experiments it has been concluded that the acid and base catalyzed enolization of ordinary ketones are general acid and base catalyzed reactions, which follow equation (1).<sup>16,17</sup> The present study of deuterations in acetate buffers is in harmony with this theory. From the pH of the buffer, the catalytic rate constants of  $D_3O^+$  and  $OD^-$  given in Ref. 14 and the amounts of total acid and base catalyzed reactions given in Table 1 it can be calculated that in expts. 2 and 3 the contribution from  $k_{D_3O^+} [D_3O^+]$  is more than one power of ten smaller than the rate of total acid catalyzed reactions. In the case of base catalyzed reactions similar calculations on expts. 1 and 2 gave contribution from  $k_{OD^-} [OD^-]$  to about five powers of ten smaller than the rate of total base catalyzed reactions. Calculations on the values given in Table 1 indicate that in the aqueous solution of acetate (expt. 5) the term  $k_{OD^-} [OD^-]$  contributes about 85 % of the total deuteration and the term  $k_{OAc^-} [OAc^-]$  only 15 %.

Table 1. Results of deuteration experiments.

Expt. No.	Molar ratio DOAc/NaOAc	Temp. °C	$k_{\text{obs}} \text{CH}_2 \times 10^7$	$k_{\text{obs}} \text{CH}_3 \times 10^7$	$K_D$	Acid catalyzed, %
1	1	60	5.7	5.4	0.71	0
2	5.4	30	4.3	3.7	0.77	20
3	21	30	2.7	1.4	1.28	67
4	84	30	2.5	0.98	1.70	84
5	0	30	6.1	5.8	0.70	0

Table 2. Composition of the runs.

Expt. No.	Solvent	DOAc ml $\times 10^{-3}$	NaOAc mg
1	0.5 ml of D <sub>2</sub> O	14.7	20.5
2	0.167 ml of D <sub>2</sub> O	333	82.0
3	0.167 ml of D <sub>2</sub> O	333	20.5
4	0.167 ml of D <sub>2</sub> O	333	5.12
5	0.5 ml of D <sub>2</sub> O	—	20.5

From the discussion above it is clear that in expt. 1 (buffer ratio 1:1), where only base catalyzed deuteration takes place, all deuteration is catalyzed by acetate ions. Therefore, the  $K_D$ -value of acetate ions can be carefully determined in this medium. The observed value, 0.70, is the same value as that given in Ref. 14 for the total base catalyzed deuteration in an aqueous acetate solution and quite different from the value given by Warkentin and Tee ( $K_D = 1.44$ ).<sup>15</sup>

However, it can be of interest to discuss why Warkentin and Tee obtained the value they did. These authors have calculated their catalytic constants by an "extrapolation technique". Dr. Tee has kindly informed me how this technique works. They considered the observed rates to be

$$k_{\text{obs}} = k_{\text{OAc}^-} [\text{OAc}^-] + k_{\text{OD}^-} [\text{OD}^-] + k_{\text{DOAc}} [\text{DOAc}] \quad (2)$$

For various concentrations of acetate ions, the values  $k_{\text{obs}}/[\text{OAc}^-]$  are plotted against

$[\text{OAc}^-]^{-1}$  and the intercepts are supposed to give the catalytic constants of acetate. These are the values given by Warkentin and Tee.<sup>15</sup>

However, a comparison of eqns. (1) and (2) shows that the method used by Warkentin and Tee neglects  $v_0$ , the catalytic power of D<sub>2</sub>O. It has been found that the deuteration of butanone-2 could be catalyzed by pure D<sub>2</sub>O, and the rate constants for the reaction determined.<sup>14</sup> These values are of the same magnitude as the value for the deuterium exchange of isobutyraldehyde-2-*d*, which was calculated by Hine *et al.* by a subtraction method.<sup>9</sup> Although these values for pure water (or D<sub>2</sub>O) were determined or calculated for 30°C or 35°C, they are less than two powers of ten smaller than the catalytic constants for acetate at 60°C given by Warkentin and Tee.<sup>15</sup>

In this connection it is of interest to note that the  $K_D$ -value for acetate given by Warkentin and Tee (1.44) is approximately the value for D<sub>2</sub>O (1.60).<sup>14,15</sup>

*Experimental.* The NMR-spectra were recorded on a Varian model A-60 spectrometer.

*Deuterations.* The composition of the different runs are given in Table 2. The reactions were performed in NMR-tubes kept at 30°C or 60°C.

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## Isolation and Identification of Prostaglandins from Ram Seminal Plasma

### Prostaglandins and Related Factors 55

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Ram semen was found in an earlier investigation by Bergström *et al.*<sup>1</sup> to contain at least two different factors with smooth muscle stimulating and blood pressure lowering effects. One of these factors showed certain properties similar to those of PGE<sub>1</sub>.

Later investigations by Samuelsson<sup>2</sup> on human seminal plasma led to identification of PGE<sub>1</sub>, PGE<sub>2</sub>, PGE<sub>3</sub>, PGF<sub>1 $\alpha$</sub> , and PGF<sub>2 $\alpha$</sub> , and more recent studies by Hamberg and Samuelsson<sup>3,4</sup> resulted in the isolation and determination of the structures of eight additional prostaglandins. These latter compounds are derivatives of the PGE compounds and can be visualized to be formed by hydroxylation at C-19 and/or dehydration involving the hydroxyl group at C-11.

The present report is concerned with the separation and identification of prostaglandins in ram semen and quantitative determination of the components using the procedure described by Bygdeman and Samuelsson<sup>5,6</sup> for determination of prostaglandin in human seminal plasma.

*Methods. Labeled prostaglandins.* Tritium labeled prostaglandin E<sub>1</sub> and F<sub>1 $\alpha$</sub>  synthesized as described earlier<sup>7,8</sup> were added to the samples in order to follow separation and recovery.

*Extraction procedure.* Samples of ram semen were obtained by using an artificial vagina and kept at -20° until being processed. To 10 ml of seminal plasma 60 ml of ethanol was added twice and the supernatants were decanted off after centrifugation. After evaporation under reduced pressure and acidification to pH 3 with N hydrochloric acid the solution was extracted three times with ether. The ether extract was washed with water until neutral reaction and then evaporated to dryness under reduced pressure.