[CONTRIBUTION FROM THE DIVISION OF BIOCHEMISTRY, MAYO FOUNDATION]

Hemihydrohalides of 3(α)-Hydroxy Steroids[1]

BY VERNON R. MATTOX, BERNARD F. MCKENZIE AND EDWARD C. KENDALL

The formation of a crystalline compound by treatment of 3(α)-hydroxy-12-keto-$\Delta^{9,11}$-cholenic acid in ether with hydrogen bromide has been observed.[2] The experimental details for preparation of this product have recently been published,[3] and the procedure was suggested as a method for separation of the cholenic acid. However, no structure was suggested for the product and no analysis was given.

In the presence of hydrogen bromide or hydrogen chloride, crystalline products have been prepared in this laboratory with the already mentioned cholenic acid and also with methyl 3(α)-hydroxy-12-ketocholanate. With both of these steroids it was found that the crystalline compounds separated in combination with 0.5 molecule of hydrogen halide.

Subsequently it was observed that, when methyl 3(α)-hydroxy-12(α)-methoxy-$\Delta^{9,11}$-cholenate was treated with hydrogen halides, the 12(α)-halogen derivative which was produced separated in crystalline form and contained in each instance 0.5 molecule of halogen acid.

The diverse nature of the material treated with hydrogen halide and the fact that only 0.5 molecule of halogen acid was combined with the crystalline product indicated that neither the double bond at C_9-C_{11} nor the substituent at C_{12} was essential. It occurred to us that it was desirable to determine what structure of the steroid was necessary for the formation of the hemihydrohalide derivatives. Such an investigation has revealed the fact that all derivatives of cholane, $\Delta^{9,11}$-cholene and Δ^{11}-cholene which have been studied (Table I) in which there was an (α)-hydroxyl group at C_3, form crystalline products and separate with 0.5 molecule of halogen acid. If the 3(α)-hydroxyl group is esterified, as with the acetyl group, addition products are not formed,[4] as shown in the last three lines of the table. No attempt has been made to prepare hemihydrohalides from 3(β)-hydroxy steroids or from steroids which have hydroxyl groups in other positions.

The general method of preparation consisted of the introduction of a stream of dry hydrogen halide into a solution containing from 1 to 3 millimoles of the compound in an appropriate solvent in an ice-bath. It was hoped that a single solvent or a mixture of solvents would be satisfactory for the isolation of all of the hemihydrohalides; however, it was found that this was not possible. For several of the methyl esters methanol was a satisfactory solvent; acetone was used for some of the acids and chloroform–petroleum ether was suitable in other cases.

The hemihydrohalides were dried under reduced pressure over sodium hydroxide or in air at room temperature for one to two days. Methyl 3(α)-hydroxy-12(α)-bromo-$\Delta^{9,11}$-cholenate hemihydrobromide retained 0.5 molecule of hydrogen bromide after it had been dried at 0.1 mm. pressure at room temperature for six hours. Most of the hemihydrohalides melt over a range of several degrees and the melting point is frequently dependent on the rate of heating. Their physical constants are given in Table I.

The halogen content of the compounds was determined by distribution between water and an immiscible solvent and titration of the halogen in the aqueous phase by the method of Volhard. In 6 experiments the amount of acid in the aqueous phase was determined. In each instance the concentration agreed with that of the halogen ion.

That no deep-seated change of the steroid molecule had occurred was shown by isolation of the starting material from the organic phase after removal of hydrogen halide. Two of the reaction products, methyl 3(α)-hydroxy-12(α)-bromo-$\Delta^{9,11}$-cholenate hemihydrobromide and methyl 3(α)-hydroxy-12(α)-chloro-$\Delta^{9,11}$-cholenate hemihydrochloride, were prepared by treatment of methyl 3(α)-hydroxy-12(α)-methoxy-$\Delta^{9,11}$-cholenate with the appropriate hydrogen halide. These two were reconverted into methyl 3(α)-hydroxy-12(α)-methoxy-$\Delta^{9,11}$-cholenate by treatment with methanol.[5,6]

It is thought that these hemihydrohalides of the 3(α)-hydroxy steroids are oxonium compounds. Favorskii[7] has prepared oxonium compounds from the aliphatic alcohols and reports that they are extremely hygroscopic. However, the oxonium compounds of the steroids reported in this paper are not hygroscopic. In addition, Favorskii also prepared from 2,2-dimethylpentanol-3 both a

(1) This paper was presented at the Fifteenth Midwest Regional Meeting of the American Chemical Society, Kansas City, Missouri, June 24, 1947.

(2) Reported by Dr. E. S. Wallis at a conference of the Committee on Synthesis of Adrenal Hormones held under auspices of the National Research Council, Washington, D. C., 1942.

(3) Hicks, Berg and Wallis, *J. Biol. Chem.*, **162**, 633 (1946).

(4) Since only the 3(α)-hydroxyl group appears to be necessary for formation of hemihydrohalides, separation of 3(α)-hydroxy-12-keto-$\Delta^{9,11}$-cholenic acid from 3(α)-hydroxy-12-ketocholanic acid cannot be made satisfactorily. For a discussion see McKenzie, Mattox, Engel and Kendall, *J. Biol. Chem.*, **173**, 271 (1948).

(5) Mattox, Turner, Engel, McKenzie, McGuckin and Kendall, *J. Biol. Chem.*, **164**, 569 (1946).

(6) When a solution of methyl 3(α)-hydroxy-12(α)-bromo-$\Delta^{9,11}$-cholenate hemihydrobromide in chloroform is repeatedly concentrated under reduced pressure and diluted with petroleum ether, methyl 3(α)-hydroxy-12(α)-bromo-$\Delta^{9,11}$-cholenate is obtained; however, because of its somewhat variable melting point, it is not satisfactory for identification. For this reason the 12-halogen compounds were converted into the 12(α)-methoxy compound, which has characteristic physical properties.

(7) Favorskii, *Chem. Abstr.*, **8**, 493 (1914).

TABLE I

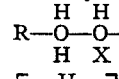
PHYSICAL CONSTANTS OF THE HEMIHYDROHALIDES

Starting material	Halogen acid	Solvent	M. p.,[a] °C.	% yield	Addition product % of 0.5 HX As prepared	After 45 days[b]
Methyl 3(α)-hydroxy-12(α)-bromo-Δ^{9,11}-cholenate[c]	HBr	$CHCl_3$-P. E.[d]	137–138	100	97[e,f]	
Methyl 3(α)-hydroxy-12(α)-chloro-Δ^{9,11}-cholenate[c]	HCl	$CHCl_3$-P. E.	131–137	94	90[f,g]	
3(α)-Hydroxy-12-ketocholanic acid	HBr	Ether	128–133	54	103	
	HCl	$CHCl_3$	115–117	86	83	62
Methyl 3(α)-hydroxy-12-ketocholanate	HBr	CH_3OH	127–130	55	98	
	HCl	CH_3OH	108–109[h]	78	99	55
3(α)-Hydroxy-12-keto-Δ^{9,11}-cholenic acid	HBr	$CHCl_3$-P. E	134–137	97	98	
	HCl	Acetone	119–122	40	95	
Methyl 3(α)-hydroxy-12-keto-Δ^{9,11}-cholenate	HBr	CH_3OH	117–123	65	96	
	HCl[i]	CH_3OH	85–90[j]	86	95	36
3(α)-Hydroxy-Δ^{11}-cholenic acid	HBr	Acetone	118–122	39	98	90
	HCl	$CHCl_3$		96	74	
Methyl 3(α)-hydroxy-Δ^{11}-cholenate	HBr	CH_3OH	93–98	81	94	80
Methyl 3(α)-hydroxycholanate	HBr	CH_3OH	115–122	45	92	
	HCl	CH_3OH	105–106	75	30	10
3(α)-Hydroxy-11-keto-24,24-diphenyl-Δ^{23}-cholene[k]	HBr	Acetone	143–150	93	99	93[l]
Methyl 3(α)-acetoxy-12(α)-chloro-Δ^{9,11}-cholenate	HCl	$CHCl_3$-P. E.			0	
3(α)-Acetoxy-12-ketocholanic acid	HCl	$CHCl_3$-P. E.			0	
3(α)-Acetoxy-12-keto-Δ^{9,11}-cholenic acid	HCl	$CHCl_3$-P. E.			0	

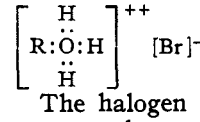
[a] All melting points were determined on the Fisher–Johns apparatus. [b] Samples were exposed to atmosphere. [c] This compound was prepared from methyl 3(α)-hydroxy-12(α)-methoxy-Δ^{9,11}-cholenate. The conversion of the 12(α)-methoxy compound to the 12(α)-halogen derivative has been shown to be nearly quantitative.[5] [d] P. E. is petroleum ether. [e] Analysis by combustion. [f] This figure is based on the halogen found in excess of that calculated for 1 atom of halogen at C_{12}. [g] For halogen determination the sample was heated in 1 *N* methanolic sodium hydroxide for ten minutes and the chloride ion was titrated by the Volhard method. [h] M. p. 112–115° when heated rapidly. [i] Crystals separated when the solution was about 2 *N* with HCl; as the solution became more concentrated with HCl the crystals dissolved. [j] M. p. 90–93° when heated rapidly. [k] Prepared from methyl 3,9-epoxy-11-ketocholanate[9] by treatment with phenylmagnesium bromide and dehydration of the resulting carbinol with acetic acid. The 3,9-epoxy linkage was opened with hydrogen bromide and the halogen was removed from C_{12} with zinc in acetic acid. [l] After 45 days in desiccator over solid sodium hydroxide.

hemihydrohalide, $(C_7H_{15}OH)_2 \cdot HBr$, and a hydrohalide, $C_7H_{15}OH \cdot HBr$. Only the hemihydrohalides of the 3(α)-hydroxy steroids could be isolated.

Favorskii represented the structure of the hemihydrohalide of the aliphatic alcohols by



However, Whitmore[8] suggested



[Br]$^-$[OR]$^-$ as being more probable.

The halogen content of some of the oxonium compounds was found to be less than the theoretical amount for a hemihydrohalide. This was observed more frequently with the hydrochlorides than with the hydrobromides and may be related to the intrinsic strengths of the two acids.

The steroid hemihydrohalides are unstable compounds and slowly lose halogen acid under atmospheric conditions. A comparison of the last two columns in the table shows the amount of halogen acid lost in forty-five days.

Summary

Crystalline oxonium compounds which contain a half molecule of hydrogen halide are formed by treatment of 3(α)-hydroxysteroids with hydrogen halides. Acetylation of the hydroxyl group prevents formation of the oxonium compound. The preparation of sixteen of these hemihydrohalides is described in this paper.

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(8) Whitmore, "Organic Chemistry," D. Van Nostrand Co., New York, N. Y., 1937, p. 139.

(9) Turner, Mattox, Engel, McKenzie and Kendall, *J. Biol. Chem.*, **166**, 345 (1946).