[Chem. Pharm. Bull. 21(4) 899—902 (1973)]

UDC 547.92.08 : 543.544

Analytical Chemical Studies on Steroids. LXIII.[1] Steroid Numbers of Androstanones and Their Oxime Derivatives on Gas Chromatography

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(Received August 31, 1972)

During the course of our studies on the metabolism of the modified steroids it has become requisite to characterize an oxygen function introduced into the steroid nucleus with a limited amount of the biotransformation product. The gas chromatographic technique has made it possible to separate microgram amount of steroids for the purpose of identification and estimation. The characterization or recognition of functional groups may be attainable by the use of parameters such as steroid number (SN),[3] T-value,[4] and methylene unit.[5] Of these proposals the SN concept appears to be particularly useful for the structural elucidation

Table I. Steroid Numbers of Androstanones

Compound	SN	SN contribution of funct. group
5α-Androstane		
1-oxo[a]	20.8	1.8
2-oxo[b]	21.2	2.2
3-oxo[c]	21.5	2.5
4-oxo[d]	21.1	2.1
6-oxo[e]	21.1	2.1
7-oxo[f]	20.9	1.9
11-oxo[g]	20.3	1.3
12-oxo[h]	21.2	2.2
15-oxo[i]	20.9	1.9
16-oxo[j]	21.2	2·2
17-oxo[k]	21.2	2.2
5β-Androstane		
4-oxo[d]	20.6	1.6
6-oxo[l]	20.7	1.7
Cholestane	27.0(15.4 min)	
Androstane	19.0(1.8 min)	

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by gas chromatography. It is also possible to characterize or detect a functional group through the utilization of reagents which alter the gas chromatographic properties of the compound upon leading to the suitable derivatives, *e.g.* O-methyloxime,[6] O-trimethylsilyloxime,[7] O-benzyloxime,[8] and N,N-dimethylhydrazone[9] for the ketone. However, the SN contributions

TABLE II. Steroid Numbers of Androstanone Oxime Derivatives

Compound	SN	SN contribution of funct. group	ΔSN[a]
5α-Androstane			
1-NOMe	20.9	1.9	0.1
2-NOMe	21.5	2.5	0.3
	21.8	2.8	0.6
3-NOMe	22.2	3.2	0.7
4-NOMe	21.7	2.7	0.6
6-NOMe	21.2	2.2	0.1
7-NOMe	21.3	2.3	0.4
12-NOMe	21.2	2.2	0
15-NOMe	21.3	2.3	0.4
16-NOMe	21.9	2.9	0.7
17-NOMe	21.8	2.8	0.6
1-NOTMS	21.6	2.6	0.8
2-NOTMS	22.3	3.3	1.1
	22.9	3.9	1.7
3-NOTMS	23.5	4.5	2.0
4-NOTMS	22.7	3.7	1.6
6-NOTMS	21.8	2.8	0.7
7-NOTMS	21.7	2.7	0.8
12-NOTMS	21.8	2.8	0.6
15-NOTMS	22.0	3.0	1.1
16-NOTMS	23.2	4.2	2.0
17-NOTMS	22.9	3.9	1.7
1-$NOCH_2Ph$	27.0	8.0	6.2
2-$NOCH_2Ph$	28.1	9.1	6.9
	29.0	10.0	7.8
3-$NOCH_2Ph$	29.2	10.2	7.7
4-$NOCH_2Ph$	28.5	9.5	7.4
6-$NOCH_2Ph$	27.6	8.6	6.5
7-$NOCH_2Ph$	27.3	8.3	6.4
12-$NOCH_2Ph$	27.4	8.4	6.2
15-$NOCH_2Ph$	27.9	8.9	7.0
16-$NOCH_2Ph$	29.3	10.3	8.1
17-$NOCH_2Ph$	29.0	10.0	7.8
5β-Androstane			
4-NOMe	20.9	1.9	0.3
6-NOMe	20.7	1.7	0
4-NOTMS	21.4	2.4	0.8
6-NOTMS	21.3	2.3	0.7
4-$NOCH_2Ph$	26.7	7.7	6.1
6-$NOCH_2Ph$	27.1	8.1	6.4
	27.3	8.3	6.6
Cholestane	27.0(15.4 min)		
Androstane	19.0(1.8 min)		

a) Expressed an increment to SN observed when the parent ketone was converted into the oxime derivative.

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of functional groups at the various positions on the steroid nucleus have not yet fully been estimated. The use of relatively simple reference sample, that is monofunctional steroid, would be preferable for obtaining the SN contribution value with more accuracy. The purpose of this paper is to record SN of androstanones and their oxime derivatives, and contributions of these functional groups to SN.

An initial effort was directed to determination of SN of 5α-androstanones possessing an oxo group at various positions. 5β-Androstan-4- and 6-ones were also taken as the test sample since these ketones are produced by the facile epimerization at C–5. The SN values were determined on a 3% SE-30 column according to the proposed definition with use of two standards, cholestane and androstane. Table I contains SN of various monoketones and SN contribution values of their oxo groups. The contribution varies with the location of a functional group on the steroid skeleton in the range of 1.3 to 2.5.

Then the chromatographic behaviors of three kinds of the oxime derivatives were examined. Almost all showed a single peak with the exception of the 5α-androstan-2-one derivatives which exhibited two peaks probably due to the formation of the *syn*/*anti* isomers. The 11-ketone which suffers from the steric hindrance to some extent failed to react with the reagent under the conditions employed. It is evident from the data listed in Table II that the SN contribution values of three oxime derivatives are greater than those of the corresponding ketones, and the magnitudes of these values have the sequence O-methyloxime<O-trimethylsilyloxime<O-benzyloxime. An increment of SN produced when the parent ketone is converted into the oxime derivative appears to be characteristic of the position. The steric hindrance involving the functional group may probably affect the SN increment to the less

Table III. Steroid Numbers of Androstanediones and Their Dioxime Derivatives

Compound	SN	
	Expected	Observed
5α-Androstane		
2,17-dioxo[a]	23.4	23.6
3,17-dioxo[b]	23.7	23.9
4,17-dioxo[c]	23.3	23.3
6,17-dioxo[d]	23.3	23.2
7,17-dioxo[e]	23.1	22.9
2,17-diNOMe	24.3	24.5
	24.6	24.7
3,17-diNOMe	25.0	25.1
4,17-diNOMe	24.5	24.5
6,17-diNOMe	24.0	23.9
7,17-diNOMe	24.1	24.1
2,17-diNOTMS	26.2	26.6
	26.8	27.2
3,17-diNOTMS	27.4	27.5
4,17-diNOTMS	26.6	26.7
6,17-diNOTMS	25.7	25.7
7,17-diNOTMS	25.6	25.5
5β-Androstane		
4,17-dioxo[f]	22.8	22.8
4,17-diNOMe	23.7	23.7
4,17-diNOTMS	25.3	25.1

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f) T. Nambara, T. Iwata, and H. Takahashi, to be published.

extent. The derivatization reaction, in particular O-trimethylsilyloxime formation, is effective to magnify the slight difference in an arrangement of the oxo group on the steroid nucleus. An extreme example of this effect can be seen in that the 4- and 6-ketones in the 5α-series are distinctly separated when transformed into the oxime derivatives. In addition, the influence of derivatization can be similarly measured in the increased separation factor for C-5 epimeric 4-ketones.

Next project was focused to determination of the SN value with 5α-androstanediones having an oxo function at C-17. The di-(O-benzyloxime) derivable from the diketone is not necessarily suitable as a derivative for gas chromatography, because it requires prolonged time for elution. The additive values obtainable from each SN contribution of two oxo groups, their O-methyloxime and O-trimethylsilyloxime are collected together with the experimental data in Table III. In general the result gave fairly good agreement between the observed and expected values. The additivity rule appears to be valid for the bifunctional oxime derivatives unless intramolecular interactions between the functional groups are involved.

It is hoped that these data will be helpful for providing a tentative structural hypothesis by the gas chromatographic method in the biochemical studies on steroids.

Experimental

Materials——All the samples employed in this work were synthesized by the established procedures in this laboratory.

Preparation of Derivatives——O-Methyloxime: To a solution of the sample (*ca.* 1 mg) in pyridine (0.5 ml) was added $NH_2OMe \cdot HCl$ (*ca.* 3 mg) and allowed to stand at 50—60° overnight. After evaporation of the solvent with the aid of a N_2 gas stream the residue was dissolved in THF (0.2 ml) and 1—2 μl aliquot was injected to gas chromatograph.

O-Trimethylsilyloxime: To a solution of the sample (*ca.* 1 mg) in pyridine (0.5 ml) was added $NH_2OH \cdot HCl$ (*ca.* 2 mg) and heated at 70—80° for 1 hr. To the resulting solution were added hexamethyldisilazane (0.2 ml) and trimethylchlorosilane (0.1 ml) and allowed to stand at room temperature for 1 hr. After evaporation of the solvent with the aid of a N_2 gas stream the residue was dissolved in THF (0.2 ml) and 1—2 μl aliquot was used for gas chromatography.

O-Benzyloxime: To a solution of the sample (*ca.* 1 mg) in pyridine (0.5 ml) was added $NH_2OCH_2C_6H_5 \cdot HCl$ (*ca.* 4 mg) and allowed to stand at 50—60° overnight. After evaporation of the solvent with the aid of a N_2 gas stream the residue was dissolved in THF (0.2 ml) and 1—2 μl aliquot was used for gas chromatography.

Gas Chromatography——The apparatus used for this work was a Shimadzu Model GC-1C gas chromatograph equipped with a hydrogen flame ionization detector and a U-shaped stainless-steel column (2.25 m × 3 mm i.d.). The column was packed with 3% SE-30 on a support of Chromosorb W (60—80 mesh). The detector and flash heater were kept at 250°, while the column was at 220°. The relative retention time of each compound was measured using cholestane as a reference compound. According to the definition proposed by VandenHeuvel and Horning[3] a plot of log relative retention time against SN was made, whereby the values of androstane and cholestane were taken as 19 and 27, respectively.

Acknowledgement This work was supported in part by a Grant-in-Aid from Iatrochemical Research Foundation, which is gratefully acknowledged.