not completely separated. Firstly it may prove impossible to obtain a complete separation by a single chromatographic fractionation. Furthermore, the active principles in the different organs of 9-day-old chick embryos could behave somewhat differently in chromatography, and overlap partially.

The results suggest complex hind-head inductions to arise from the cooperation of the neural- and the mesodermal-inducing factors. SAXÉN AND TOIVONEN[4] came to a similar conclusion by testing heated (archencephalic-inducing) and non-heated (spinocaudal-inducing) HeLa cells together. Combination experiments with mesodermal and archencephalic neural-inducing fractions have furnished new arguments for this hypothesis*.

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Gas-chromatographic characterization of steroid ketones as *N,N*-dimethylhydrazones

Gas-chromatographic techniques have made it possible to separate microgram or submicrogram amounts of many steroids for purposes of identification and estimation. The identification or recognition of functional groups may be made by the determination of "steroid numbers"[1] (summations of values determined by the carbon content of the steroid nucleus and values characteristic of the functional groups of the steroid), by the determination of "*T* values"[2] (based on changes in retention-time behavior observed with changes in the liquid phase) and by the use of ΔR_{Mg} values[3] (by analogy with paper partition chromatography). It is equally possible to characterize or detect functional groups through the use of reagents which alter the gas-chromatographic properties of the compounds under study through derivative formation. This is not different in principle from the use of classical functional-group reagents to detect or identify specific functional groups, but the requirements for the reaction products are different. Classical techniques usually provide relatively insoluble crystalline

Abbreviations: SE-30, methyl silicone polymer; QF-1, fluorinated alkyl silicone polymer (10000 cs); CNSi, cyanoethylmethylsilicone polymer (65 mole % cyanoethyl).

TABLE I

RELATIVE (TO CHOLESTANE) RETENTION TIMES FOR STEROID DIMETHYLHYDRAZONES

Dimethylhydrazones were made by dissolving the steroid in anhydrous *N,N*-dimethylhydrazine; in specified instances glacial acetic acid was used as a catalyst. Complete reaction required 1–2 h at room temperature. The 3-dimethylhydrazone of androstane-3,17-dione was prepared by dissolving a sample of androstane-3,17-dione in *N,N*-dimethylhydrazine, and removing after 2 h the excess reagent in a stream of N_2. Gas-chromatographic analysis of the sample indicated complete conversion to the 3-dimethylhydrazone. In independent studies it was observed that a carbonyl group in the 17-position will not undergo reaction under these conditions. The nature of the reaction was confirmed by infrared analysis (the presence of a band in the infrared spectrum corresponding to a 5-membered ring ketone, and the lack of a band corresponding to a 6-membered ring ketone, indicated that the 17-one group was still present) and by elementary analysis (Calc. for $C_{21}H_{34}ON_2$: C, 76.29; H, 10.37; N, 8.47%. Found: C, 76.53; H, 10.47; N, 8.71%. m.p. 111–114°.). The 3,17-di-(dimethylhydrazone) of androstane-3,17-dione was prepared by dissolving a sample of androstane-3,17-dione in *N,N*-dimethylhydrazine (acetic acid catalyst) and removing after 2 h the excess reagent in a stream of N_2. Gas-chromatographic analysis of the sample indicated complete conversion to the 3,17-di-(dimethylhydrazone). The nature of the reaction was confirmed by infrared analysis (the absence of carbonyl bands in the infrared spectrum) and by elementary analysis (Calc. for $C_{23}H_{40}N_4$: C, 74.14; H, 10.82; N, 15.03%. Found: C, 74.02; H, 10.95; N, 15.26%. m.p. 143–146°.). A sample of this material was collected after chromatography and found to be unchanged. The 20-dimethylhydrazone of 5α-pregnane-3β-ol-11,20-dione was prepared by dissolving a sample of 5-pregnane-3-ol-11,20-dione in *N,N*-dimethylhydrazine (acetic acid catalyst) and removing after 12 h the excess reagent in a stream of N_2. Gas-chromatographic analysis of the sample indicated that only one ketone group had undergone reaction. In independent studies it was observed that a carbonyl group in the 20-position will undergo reaction under these conditions, and the conclusion was drawn that the carbonyl band (characteristic of a 6-membered ring ketone) found in the infrared spectrum was due to the 11-keto group. Elementary analysis confirmed that the compound was a mono-dimethylhydrazone (Calc. for $C_{23}H_{38}O_2N_2$: C, 73.75; H, 10.23; N, 7.48%. Found: C, 73.71; H, 10.31; N, 7.42%. m.p., 65–67°). The 3-dimethylhydrazones of cholestane-3-one and 4-cholestene-3-one were isolated and had satisfactory elementary analyses. For the derivative of cholestane-3-one, $C_{29}H_{52}N_2$, calc.: C, 81.24; H, 12.23; N, 6.53%; found: C, 81.51; H, 12.27; N, 6.71%. m.p., 103–105°. For the derivative of 4-cholestene-3-one, $C_{29}H_{50}N_2$, calc.: C, 81.63; H, 11.81; N, 6.56%; found: C, 81.67; H, 11.73; N, 6.44%. This compound is a pale yellow highly viscous liquid. Other derivatives were prepared by the following short procedure. A 0.1–1.0 mg quantity of steroid was dissolved in 0.1–0.2 ml of *N,N*-dimethylhydrazine, and about 0.05 ml of acetic acid was added if necessary. The excess reagent was removed by a stream of N_2 after an appropriate reaction time. The residue was dissolved in redistilled tetrahydrofuran and the solution was used directly for chromatographic work. Hydroxyl groups were not affected by this procedure. The column conditions were as follows. SE-30 stationary phase: 1% SE-30 on 100–140 mesh Gas-Chrom P; 6 ft × 4 mm glass U-tube; 215°; 16 lb/in²; cholestane time 13.5 min. QF-1 stationary phase: 1% QF-1 on 100–140 mesh Gas-Chrom P: 6 ft × 5 mm glass U-tube; 210°; 10 lb/in²; cholestane time 3.1 min. CNSi stationary phase: 2% CNSi on 100–120 mesh Gas-Chrom P; 6 ft × 4 mm glass U-tube; 215°; 17 lb/in²; cholestane time, 3.4 min. A gas chromatograph equipped with an argon-ionization detector was used throughout the investigation.

Compound	*Relative retention times*								
	SE-30			*QF-1*			*CNSi*		
	Free	*DMH**	*DMH + HOAc***	*Free*	*DMH*	*DMH + HOAc*	*Free*	*DMH*	*DMH + HOAc*
Androstane-17-one	0.19	0.19	0.27	0.64	0.64	0.39	0.81	0.81	0.48
Androstane-3,17-dione	0.42	0.61	0.85	4.06	2.00	1.17	6.76	3.48	1.93
Androstane-3β-ol-16-one	0.38	0.38	0.63	2.47	2.47	1.49	5.03	5.03	3.38
5α-Pregnane-3,20-dione	0.68	0.99	1.57	5.51	2.84	1.97	8.47	4.57	3.04
5α-Pregnane-20β-ol-3-one	0.70	1.00	1.00	3.49	1.90	1.90	6.79	3.79	3.79
5α-Pregnane-3β-ol-20-one	0.64	0.64	1.02	2.80	2.80	1.92	5.96	5.96	3.87
5α-Pregnane-3β-ol-11,20-dione	0.88	0.88	1.52	7.16	7.16	5.10	20.0	20.0	12.8
Cholestane-3-one	2.20	3.24	3.24	6.12	3.32	3.32	7.15	4.22	4.22
Coprostane-3-one	2.04	2.78	2.78	5.56	2.89	2.89	6.49	3.61	3.61
4-Cholestene-3-one	2.74	2.74	3.64	9.65	9.65	4.16	11.5	11.5	5.41

* DMH, *N,N*-dimethylhydrazine alone was used as the reagent.
** DMH + HOAc, acetic acid was used as a catalyst in the reaction.

products, often colored, which are formed in high yield and which may be purified easily. In gas-chromatographic work it is desirable that reaction products should be formed in high yield and the retention time of the product should be different from that of the starting material. Examples of derivatives suitable for the characterization of hydroxyl-substituted compounds are trimethylsilyl ethers[4-7] and trifluoroacetates[8]. Methods for the characterization of ketones have not yet been reported. We have therefore investigated the use of *N,N*-dimethylhydrazine as a specific reagent for the carbonyl group, with particular reference to steroid ketones.

The gas-chromatographic properties of a group of steroid dimethylhydrazones are given in Table I. The relative (to cholestane) retention times of the derivatives with a non-selective phase, SE-30 (obtained from General Electric Co.), are somewhat greater than those of the parent steroids. With the selective phases QF-1 (obtained from Dow Corning Corp.) and CNSi (obtained from General Electric Co.), the derivatives were eluted before the parent steroids. It was also observed that the separation factor for the *cis–trans* isomers coprostane-3-one and cholestane-3-one was increased after dimethylhydrazone formation. With CNSi phase the separation factor for the ketones was found to be 1.10, while a value of 1.17 was observed for the derivatives.

The condensation reaction was found to be dependent upon the position of the ketone group and upon the reaction conditions. A quantitative reaction was observed at room temperature within 1–2 h for a ketone group at the 3- position, but if the carbonyl group was conjugated with a double bond a catalytic amount of acetic acid was necessary for reaction. A carbonyl group at the 20- position was found to react in quantitative fashion within 1–2 h only if an acid catalyst was present. The condensation with 16- and 17-ones also required an acid catalyst. An 11-keto group failed to react after 12 h. The formation or non-formation of these derivatives under different conditions, detected by gas-chromatographic techniques, may therefore be used as a test for determining the number and the nature of reactive ketone groups present in a steroid (see Fig. 1).

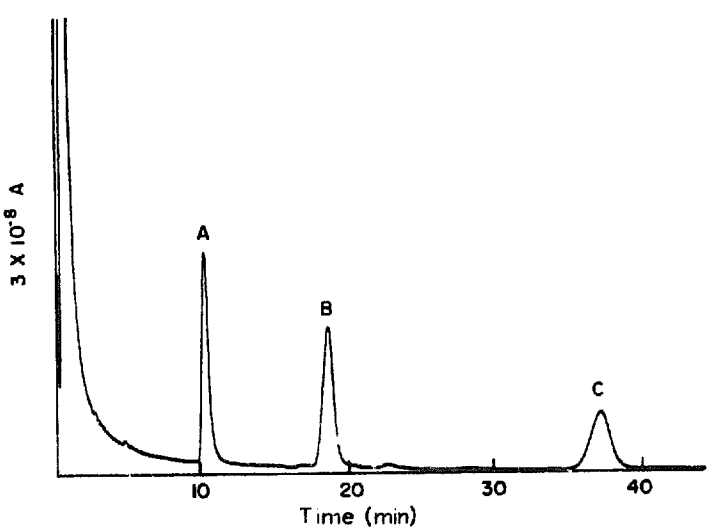

Fig. 1. Gas-chromatographic separation of a mixture of androstane-3,17-dione and two of its derivatives. The compound at A is the 3,17-di-(dimethylhydrazone) (*N,N*-dimethylhydrazine and acetic acid catalyst); the peak at B is due to the 3-dimethylhydrazone (*N,N*-dimethylhydrazine alone); the peak at C is due to androstane-3,17-dione. Each peak represents 1–2 μg of steroid. Column conditions: 6 ft × 4 mm glass U-tube; 1% QF-1 on 100–120 mesh Gas-Chrom P; 202°; 19 lb/in^2.

The results obtained with these techniques, when combined with those previously observed in studies of hydroxyl-substituted steroids[4-8], indicate that a new approach to qualitative organic chemistry and functional group analysis may be possible through utilization of gas-chromatographic methods. The ability to use microgram samples of steroids is a particular advantage of these procedures and makes them especially valuable for biochemical studies.

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