Hydrocarbons with the Odor of 5α-Androst-16-en-3-one

Compounds having the odor of 5α-androst-16-en-3-one [androstenone (1), Figure 1] play a significant role in chemoreception studies. Their odor for humans has been qualified as a primary odor on the basis of specific anosmia distribution data (Amoore, 1982). Therefore, the existence of a specific androstenone olfactory receptor has been postulated (Amoore, 1982; Wysocki and Beauchamp, 1984; Ohloff, 1986). Biochemical studies indicate the existence of an androstenone receptor in the pig olfactory tissue (Gennings et al., 1977; Hancock et al., 1985; Kraevskaya et al., 1987; Parfenova and Minor, 1989). It was also found that androstenone is responsible for boar taint (Patterson, 1968).

A carbonyl functional group in odorant molecules is considered to be a necessary structural element for many aldehydes and ketones to have their odors, because an alteration of this group by reduction to a methylene, alcohol, or ether functional group leads to significant change of odor (Beets, 1978; Ohloff, 1986). The essential role of the carbonyl group has been suggested to result from a necessary interaction with protein olfactory receptors to form a Schiff base with an amino group (Mason and Clark, 1984).

All previously known compounds having an androstenone-like odor contain the carbonyl group, whereas related compounds without this functional group have different odor types or are odorless (Beets, 1978; Ohloff et al., 1983; Zinkevich, 1988). It has also been demonstrated that stereochemical factors are critical for ketones to have this type of odor (Theimer et al., 1977; Ohloff et al., 1983; Aronov and Zinkevich, 1993). The usual methods of synthetic carbonyl group modification, such as reduction of this group to methylene or to secondary alcohol, significantly change molecular shape, which may be a reason for odor transformations. Using a computer-aided molecular modeling technique, we found that substitution of carbonyl oxygen with methylidene (=CH_2) group results in a minimal change of molecular shape (no changes in distances and angles except the van der Waals radius of the introduced CH_2 group and the CH_2=C bond length).

Thus, the main question of the present study is whether it is possible to keep the original androstenone odor in spite of substitution of the carbonyl oxygen by a methylidene (=CH_2) group. To answer the question, we obtained and tested for odor quality methylidene derivatives of androstenone (1) (Figure 1), its closest structural and functional analogue, 5α-androstan-3-one (2), and the most active synthetic androstenone-like odorant 4-(4,4-dimethylcyclohexyl)-2-methylcyclohexanone (3) (Aronov and Zinkevich, 1993).

MATERIALS AND METHODS

Chemicals and Solvents. 5α-Androst-16-en-3-one (1) and 5α-androstan-3-one (2) were obtained from Sigma; 4-(4,4-dimethylcyclohexyl)-2-methylcyclohexanone (3) was synthesized in the laboratory according to a procedure described before (Aronov and Zinkevich, 1993). All other solvents and chemicals were obtained from Aldrich. Commercial anhydrous tetrahydrofuran (THF) was used for the Wittig reaction.

Instrumentation. The liquid chromatographic system used consisted of a Gilson 305 pump, a Gilson 121 refractive index detector, and a Rheodyne injector. The gas chromatograph was a Varian 3300 equipped with a DB-5 (0.53 mm × 15 m) capillary column and a flame ionization detector. ^{1}H NMR spectrometry was performed using a Bruker WM-250 (250 MHz) instrument in $CDCl_3$ solutions. Mass spectra were obtained using LKB-9000 GS/MS system. IR spectrophotometry was carried out on a Shimadzu IR-435 spectrophotometer on 1% solutions in CCl_4.

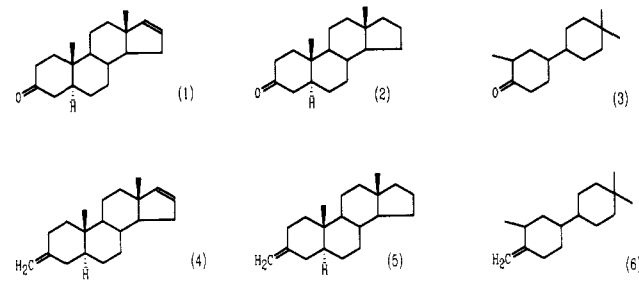

Figure 1. Structural formulas of ketones 1–3 having the androstenone odor and their methylidene derivatives: 3-methylidene-5α-androst-16-ene (4), 3-methylidene-5α-androstane (5), and 3-methyl-4-methylidene-1-(4,4-dimethylcyclohexyl)cyclohexane (6), respectively.

Methylidene Analogues of Ketones 1–3 (General Procedure). A 1:1 mixture of methyltriphenylphosphonium bromide and sodium amide (300 mg, 0.82 mmol) was added into THF (1 mL) and stirred for 1 h at room temperature; a solution of the appropriate ketone (1–3) (0.5 mmol) in 2 mL of THF was added, and the mixture was stirred for 1 h. Then water (5 mL) was added, and the mixture was extracted with hexane (3 × 10 mL). The hexane extract was filtered through a short column, packed with 1 g of silica gel, the column was additionally washed with 10 mL of hexane, and the combined filtrate was evaporated. The residue was purified by HPLC (Zorbax Sil 4.6 × 250 mm, hexane, 2 mL/min) portionwise (10 mg per injection), and homogeneity of the material obtained was confirmed by analytical HPLC (Zorbax ODS 4.6 × 250 mm, acetonitrile–water 9:1, 1.5 mL/min) and GC [100 °C (5 min), 5 °C/min to 200 °C]. The yields varied from 53 to 84%.

Odor Evaluation. The odor was estimated by eight subjects chosen by the criterion of sensitivity to the odor of androstenone; i.e., their olfactory thresholds to androstenone were determined as 1 ng according to the procedure described elsewhere (Kagan et al., 1981; Aronov and Zinkevich, 1993). The subjects could easily recognize this odor even in complex mixtures of odorants, and they repeatedly participated in psychophysical tests with this odortype. A solution of the compound to be tested (1 mg in 0.5 mL of hexane) was injected into the HPLC system (Zorbax Sil 4.6 × 250 mm column; hexane, 2 mL/min; refractive index detector); at the output of the detector three fractions of eluate were collected: mobile phase before peak, a fraction corresponding to the peak, and a fraction corresponding to baseline 1 min after the peak. Each fraction (50 μL) was applied to a filter paper strip (1 × 10 cm), and the hexane was allowed to evaporate. The compound was concluded as having the odor of androstenone only if both before and after peak fractions were determined by panelists as odorless and the odor of the substance from the fraction collected at the center of the peak was recognized as androstenone.

RESULTS AND DISCUSSION

A conversion of ketones 1–3 into corresponding methylidene derivatives was performed by the Wittig reaction. The resulting hydrocarbons (4–6) were purified by normal-phase HPLC, and the homogeneity of the purified derivatives was confirmed by reversed-phased HPLC and capillary GC. On the basis of results of GC analysis, the amount of starting material in purified hydrocarbons 4–6 was undetectable, that is, less than 0.01%. In the mass spectra of the obtained compounds intense signals were observed corresponding to the molecular ions of 4–6, and types of fragmentation corresponded to the expected ones. In the IR spectra there were no absorption bands assignable to carbonyl groups,

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while in the ^1H NMR spectra there appear signals of two olefinic protons, which support the structures of the hydrocarbons 4–6.

To avoid error in the estimation of the quality of the odor, associated with the trace contamination by the initial ketone and/or products of oxidation by air, we have used the instant purification technique, which is based on the observation that ketones, epoxides, and alcohols are retained substantially longer than corresponding hydrocarbons in chromatography on silica columns in hexane.

Organoleptic tests of hydrocarbons 4 and 6 revealed that these compounds have an odor indistinguishable from the odor of androstenone (1) for all eight subjects, while compound 5 did not have this odor for any of the subjects and was found to be odorless for most (seven) of them. Thus, the replacement of the carbonyl oxygen in compounds 1 and 3 by the methylene group did not change the odor type.

As discussed above, the molecular geometry of hydrocarbons 4–6, as compared with that of initial ketones 1–3, is substantially unchanged, whereas the distribution of the electron density and chemical properties of the substances, including the capacity to form Schiff bases, differ fundamentally. Thus, it has been revealed that the carbonyl group is not necessary for the origin of the androstenone-like odor. This means that the model based on the formation of a covalent bond of the carbonyl compounds with the receptor, in particular Schiff base linkage, does not apply to the substances with the androstenone odor.

Why does hydrocarbon 5 obtained from 5α-androstan-3-one (2) not have the above odor in contrast to 4 and 6? Androstenone (1) and 4-(4,4-dimethylcyclohexyl)-2-methylcyclohexanone (3) are the most active odorants of this type. Their thresholds were determined as 1 and 0.1 ng, respectively, whereas the threshold of 5α-androstan-3-one (2) under the same conditions is 10 ng (Aronov and Zinkevich, 1993). Apparently, there is an upper limit of a carbonyl precursor olfactory threshold, which allows retention of the original odor when the carbonyl functional group is substituted by a methylidene one. Additional experiments would be required to substantiate this observation.

We expect that the property of some methylidene hydrocarbons to retain the odor of their carbonyl analogues may be also found in compounds with a different type of odor.

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