

of sugar pyrolysis and condensation and taking into account the fact that protracted heating can remove terpenes. Twenty-eight compounds identified in unheated licorice are reported in Table II for comparison.

In conclusion, we can say that none of the identified compounds is alone responsible for licorice flavor, although many taste panels have been made on fractions, e.g., at the gas chromatographic column exit. Total extract, instead, shows a typical licorice aroma, indicating that this may be due to an integrated response to the proper mixture of the proper volatiles, rather than to the odor of one or two components.

LITERATURE CITED

- Frattini, C., Bicchi, C., Barettoni, C., Nano, G. M., *Riv. Ital. Essenze, Profumi, Piante Off. Aromi, Saponi, Cosmet., Aerosol* 58, 132 (1976).
- Frattini, C., Bicchi, C., Nano, G. M., "Mass Spectrometric Studies on Aromatic Volatile Components in Edible Plants", Japanese-Italian Symposium on Mass Spectrometry in Chemistry, Biochemistry, and Medicine, Istituto Mario Negri, Milan, Italy, Sept 1976.
- Frattini, C., Bicchi, C., Nano, G. M., *Riv. Ital. Essenze, Profumi, Piante Off.* 11(3), 597 (1974).
- Haensel, H., *Pharm. Zentralhalle Dtschl.* 40, 533 (1899).
- Isaev, V., *Acta Horti Bot. Tadshikistan*, 17 (1932); *Chem. Abstr.* 28, 3179 (1934).
- Van Straten, S., De Beauveser, J. C., De Vrijer, F., Central Institute for Nutrition and Food Research TNO, Supplement 1-8, Zeist, Netherlands, 1976.
- Van Straten, S., De Vrijer, F., Central Institute for Nutrition and Food Research TNO, Report No. 4030, 3rd ed, Zeist, Netherlands, 1973.

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Quantitative Determination of 5 α -Androst-16-en-3-one by Gas Chromatography-Mass Spectrometry and Its Relationship to Sex Odor Intensity of Pork

Raymond H. Thompson, Jr.¹ and A. M. Pearson*

Chemical analysis for determining the level of 5 α -androst-16-en-3-one by an isotope dilution/carrier technique utilizing selected ion monitoring (SIM) mass spectrometry is described. Levels of 5 α -androst-16-en-3-one were correlated with sex odor scores from a meat packing house panel and a selected laboratory panel, which resulted in "r" values of 0.27 and 0.46, respectively. Only the latter value was statistically significant ($P < 0.05$). The low relationships between odor scores and actual levels of 5 α -androst-16-en-3-one lend support to the theory that other C₁₉- Δ^{16} steroids may contribute to sex odor in pork.

Meat from sexually mature boar (uncastrated male) pigs frequently gives off an offensive odor upon heating, which has been described as "urine-like" or "perspiration-like" (Craig et al., 1962). This odor not only occurs in the meat from boars and cryptorchids but has been noted to a lesser extent in the meat from sows, barrows, and gilts (Williams et al., 1963). Bishop (1969) has suggested that the low incidence of the undesirable odor in the meat from females (sows and gilts) and in castrated males (barrows) may be due to the presence of intersexes and cryptorchids, whereas, Sink (1967) has discussed other possibilities including the stage of estrus in sows and gilts, castration, and adrenal hypertrophy.

Patterson (1968) isolated 5 α -androst-16-en-3-one from boar fat and concluded that it is responsible for the offensive odor, which confirmed the suggestion of Sink (1967) that the C₁₉- Δ^{16} steroids may be responsible for sex odor in pork. Beery et al. (1971) and Thompson et al. (1972) also verified the contribution of 5 α -androst-16-en-3-one

to sex odor in pork and presented evidence for the involvement of other C₁₉- Δ^{16} steroids. Although the relative importance of 5 α -androst-16-en-3-one and the other C₁₉- Δ^{16} steroids has not been resolved, Canadian governmental meat inspection regulations require condemnation of all boar and stag carcasses, whereas, USDA (1973) regulations specify that carcasses with "slight odor" can be used in comminuted sausages and those with "strong odor" must be condemned. Obviously, the problem of meat inspection and packing house personnel properly identifying the level of sex odor in the carcasses and their ability to relate it to the amount of 5 α -androst-16-en-3-one in the fatty tissues is an important one from the regulatory standpoint. Kloek (1961) has shown that there is wide variation in the ability of human subjects to smell different steroid hormones. More recently, Griffiths and Patterson (1970) have demonstrated some people are unable to smell 5 α -androst-16-en-3-one, whereas, others vary widely in their olfactory reaction to this compound, with some finding it pleasant and others extremely nauseating.

Since cryptorchid pigs may be frequently encountered in the normal population of slaughter hogs, it seemed desirable to see if meat inspectors and packing house personnel could relate sex odor scores of cryptorchid pigs with the levels of 5 α -androst-16-en-3-one present in the fatty tissues. Thus, the present study involved a comparison of the results from packing house olfactory tests

Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824.

¹Present address: Beltsville Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, Nutrition Institute, Beltsville, Maryland 20705.

with the actual levels of 5 α -androst-16-en-3-one as determined by chemical analysis by using an isotope dilution/carrier technique, which utilizes selected ion monitoring (SIM) mass spectrometry (Gordon and Frigerio, 1972; Sweeley et al., 1977). The same samples were also submitted to a small highly selected panel in our laboratory for olfactory scoring. Some details of the methodology and the correlation coefficients between sex odor scores for the two panels and the actual levels of 5 α -androst-16-en-3-one in the fatty tissues are presented.

MATERIALS AND METHODS

Fat Samples and Rating for Sex Odor. Packing house personnel (three-five panelists, including the USDA meat inspector and plant supervisory personnel) removed subcutaneous fat samples from cryptorchid carcasses on the slaughter line. A consensus rating for sex odor was determined on heating the fat with a hot (approximately 100 °C) iron and rating on a scale from 0 to 6 as follows: no sex odor = 0; very slight sex odor = 1; slight sex odor = 2; moderate sex odor = 3; slightly pronounced sex odor = 4; pronounced sex odor = 5; and very pronounced sex odor = 6. Using this scoring system, a total of 17 samples were obtained with distribution over the entire range of odor intensity and were related to the chemically determined levels of 5 α -androst-16-en-3-one in the fatty tissues. A portion of each original subcutaneous fat sample was packed in dry ice and shipped to our laboratory by air freight. Upon arrival, all samples were assigned random numbers for identification and stored at -20 °C until removed for subsequent analysis.

On removal of the samples from freezer storage, approximately 50 g of each sample was placed in a 200-mL beaker, covered with a watch glass, and heated to approximately 100 °C on an electric hot plate under an evacuating fume hood. The samples then were presented to three panel members from our laboratory, all of whom had previously been shown to be very sensitive to sex odor in pork. The panel members rated the samples on a 10-point scale with a score of 0 assigned for no sex odor and a score of 9 assigned to samples with very pronounced sex odor. The panel scores for sex odor were then related to the chemically determined levels of 5 α -androst-16-en-3-one in the fatty tissues by calculating the correlation coefficients (Snedecor, 1956).

Assay for 5 α -Androst-16-en-3-one. Saponification and preparative thin-layer chromatography were employed to isolate fractions suitable for GC-MS (gas chromatography-mass spectrometry) analysis. Saponification of the fatty tissue samples was accomplished by modification of the procedure of Bunnel (1967) in which a 2-g sample was placed in a 100-mL round-bottom boiling flask and a known amount of deuterium-labeled 5 α -androst-16-en-3-one (usually 21 μ g) was added and followed by addition of 15 mL of ethanol, 100 mg of pyrogallol, and a few boiling chips. The flask was connected to a reflux condenser and the mixture was refluxed for 5 min to displace the air from the flask and minimize oxidation. Approximately 1 g of KOH pellets was added so as to not interrupt refluxing, which was continued for 30 min. The flask was cooled in ice, and the contents were quickly transferred to a separatory funnel containing 15 mL of hexane and 15 mL of water, after which the mixture was shaken for 1 min. The hexane extract was removed and saved. Extraction was repeated twice using 8-mL portions of hexane. The combined extracts were washed five times with water, followed by drying over anhydrous sodium sulfate. The hexane extract was evaporated to dryness and the residue was redissolved in 250 μ L of ethyl acetate and stored at

-10 °C in a desiccator until removed for GC-MS analysis.

Preparative thin-layer chromatography was performed on "preabsorption" silica gel thin layer plates (Type PLQF, 1 mm thick, 20 $\times$ 20 cm, Quantum Industries). The sample was applied to the center of the plates and an authentic solution of 5 α -androst-16-en-3-one was applied on the edge of the plates to serve as a marker. The plates were developed in benzene-ethyl acetate (95:5) in a tank under saturated conditions. After development, the center of the plate was covered with a glass strip to protect the sample and the edge of the plate was sprayed with a mixture of sulfuric-acetic acid (1:1) to visualize the standard. The appropriate region of the plate was scraped off and placed in a small fritted glass filter funnel (no. 36060, 2 mL, medium porosity, Corning Glass Works). The sample was eluted from the silica gel by 1 h continuous extraction with four 1 mL volumes of chloroform-methanol (2:1) as recommended by Vandenheuvél (1967). The extracts were collected in a 5-mL Pierce Reactival (Pierce Chemical Co.) and evaporated to dryness in a stream of nitrogen gas. The residue was dissolved in 50 μ L of ethyl acetate and stored in a desiccator over anhydrous sodium sulfate at -10 °C until removed for GC-MS analysis.

Purification procedures were monitored with a GC-4 Beckman gas chromatograph equipped with a flame ionization detector. Silanized glass columns (183 $\times$ 0.2 cm i.d.) packed with 3% OV-1 or 5% SP-2401 on 100/120 mesh Supelcoport (Supelco, Inc., Bellefonte, Pa.) were used. Helium carrier gas was maintained at a flow rate of 22 mL/min. Hydrogen and oxygen flow rates were maintained at 60 and 300 mL/min, respectively. Additional carrier gas (helium) was introduced into the hydrogen ahead of the detector at a flow rate of 45 mL/min to improve the signal to noise ratio of the detector. The temperature parameters were 185 °C for the column, 220 °C at the inlet, and 240 °C at the detector using isothermal operating conditions. The purified samples dissolved in ethyl acetate were injected directly onto the column and compared directly with authentic standard 5 α -androst-16-en-3-one.

Quantitation of 5 α -androst-16-en-3-one in the fatty tissue extracts was done by isotope dilution/carrier techniques using SIM mass spectrometry, a technique introduced by Sweeley et al. (1966) and Hammar et al. (1968), and first used for assaying biological material by Samuelsson et al. (1970) and by Gaffney et al. (1971). For this procedure the mass spectrometer is coupled with an acceleration voltage alternator unit designed for computer control of fine focusing, data acquisition, reduction, and display (Holland et al., 1973). Quantitation was achieved by plotting the ratio of the peak areas for the molecular ions of unlabeled (m/e 272) and labeled (m/e 274) 5 α -androst-16-en-3-one against the amount of the labeled form initially added to each sample.

Combined GC-MS was carried out on an LKB-9000 instrument interfaced to a PDP-8/I minicomputer (Digital Equipment Co.) for data acquisition and reduction (Sweeley et al., 1970). The silanized glass column (183 $\times$ 0.2 cm, i.d.) was packed with 5% SP-2401 on 100/120 mesh Supelcoport (Supelco, Inc., Bellefonte, Pa.). The GC operating conditions and temperatures of operation were identical with those described for the Beckman GC-4. Mass spectral measurements were recorded at 70 eV ionization energy with full accelerating voltage of 3.5 kV and 60 μ A trap current and an ion source temperature of 250 °C. Calibration curves for the quantitative experiments were prepared by adding solutions of unlabeled and deuterated 5 α -androst-16-en-3-one in ethyl acetate to give

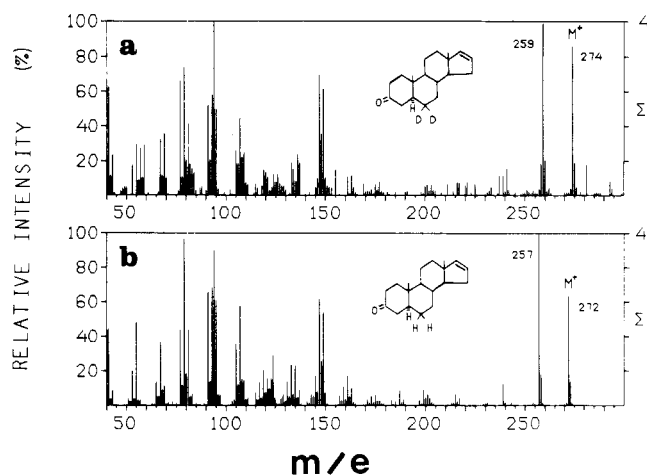


Figure 1. Mass spectra of deuterium-labeled (a) and unlabeled (b) 5 α -androst-16-en-3-one.

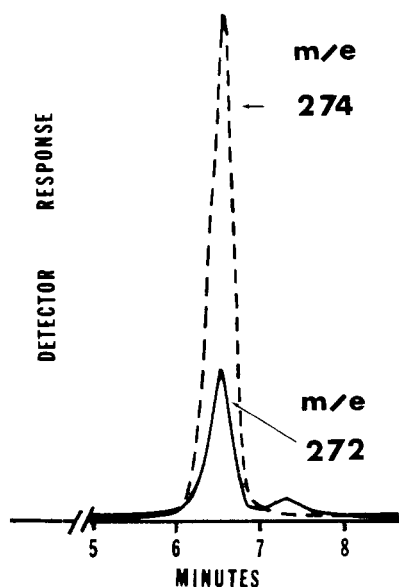


Figure 2. Typical ion intensity recording for labeled (m/e 274) and unlabeled (m/e 272) 5 α -androst-16-en-3-one.

concentrations of 0.02 and 0.20 mg/mL, respectively. Vials were prepared containing 0.01, 0.02, 0.04, 0.06, 0.08, and 0.10 mL of the unlabeled standard solution and 1 mL of the labeled standard (5 α -androst-16-en-3-one-6,6'- d_2) was added. An aliquot (1–2 μ L) was then injected into the GC-MS computer system and monitored by the SIM procedure.

RESULTS AND DISCUSSION

SIM GC-MS Technique. The SIM GC-MS technique records the intensity of preselected ions in the mass spectrum of the unlabeled compound and the deuterium-labeled analogue which serves as an internal standard and carrier. Chemical and manipulative losses occurring during isolation and analysis of 5 α -androst-16-en-3-one are automatically corrected since the ratio of the labeled and unlabeled forms should remain the same. The deuterium labeled form of 5 α -androst-16-en-3-one is an ideal quantitative standard because it is virtually identical with the unlabeled form and yet easily distinguishable by its different mass. Synthesis of the deuterium-labeled 5 α -androst-16-en-3-one will be discussed in a subsequent publication.

The mass spectra of the unlabeled and deuterium labeled 5 α -androst-16-en-3-one can be compared in Figure 1. Their molecular ions appearing at m/e 272 and 274

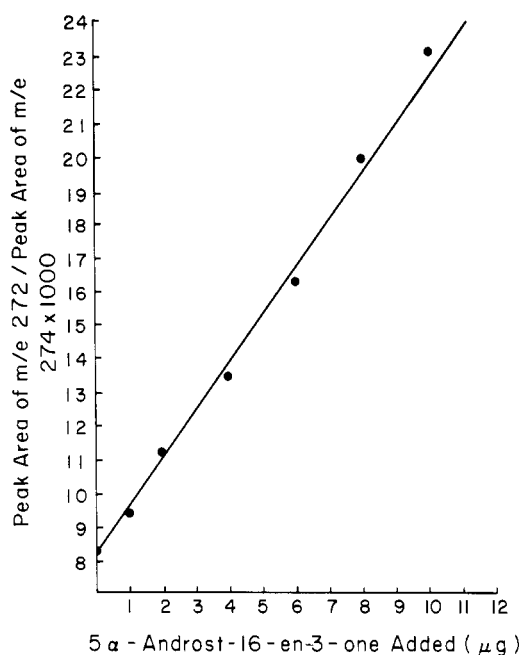


Figure 3. Calibration curve for quantification of 5 α -androst-16-en-3-one.

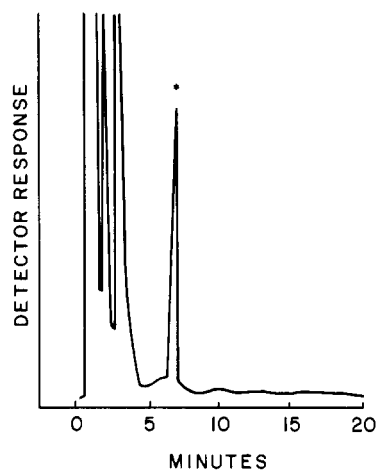


Figure 4. Gas chromatogram showing unlabeled 5 α -androst-16-en-3-one co-isolated with deuterium-labeled internal standard. The asterisk represents both labeled and unlabeled compound.

are intense ions in the spectra, which make them good ions to monitor. Figure 2 shows a typical ion recording for m/e 272 and 274 obtained from SIM GC-MS analysis of a mixture of the unlabeled and labeled forms of 5 α -androst-16-en-3-one.

Calibration Curves. The ratio between the peaks for m/e 272 (unlabeled 5 α -androst-16-en-3-one) and m/e 274 (deuterium labeled 5 α -androst-16-en-3-one) for standard mixtures of the unlabeled compound with 0.2 mg of the deuterated compounds was found to be linear with the amount of unlabeled compound added. The ratio between the peak heights at m/e 272 and 274 did not pass through the origin as can be seen in Figure 3. This was expected since the labeled compound gives a small peak at m/e 272 which is regarded as background.

Quantitative Analysis of 5 α -Androst-16-en-3-one Using the Deuterium-Labeled Standard. The purification procedure, which involved saponification and GLC (gas-liquid chromatography) resulted in a relatively pure sample with 5 α -androst-16-en-3-one eluting at about 7 min (Figure 4) on the gas chromatogram. The peak contained both the unlabeled form of 5 α -androst-16-en-3-one ori-

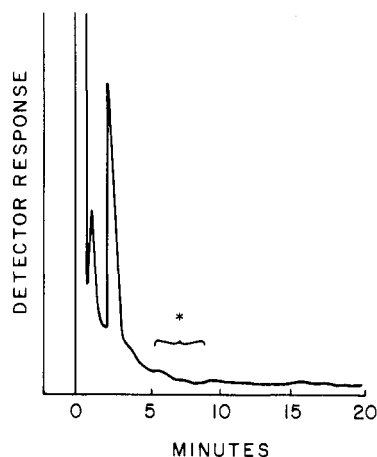


Figure 5. Gas chromatograph showing unlabeled 5α -androst-16-en-3-one isolated without deuterated internal standard. The asterisk indicates area where unlabeled peak should have appeared.

ginating from the pig fatty tissue and the d_2 form which was added as the internal standard and carrier. Figure 5 shows the GLC trace obtained for one sample to which the labeled standard was not added. There is no measurable response at 7 min retention time, indicating the absence of 5α -androst-16-en-3-one. It is evident that ordinary GC techniques without the labeled compound would not permit the assay of this sample by the GC-MS technique. The same sample was easily assayed by combined GC-MS, when excess d_2 -labeled 5α -androst-16-en-3-one was added as a carrier and internal standard. The sample (no. 88651, Table I) contained 5α -androst-16-en-3-one at a level of 0.42 $\mu\text{g/g}$ of fat.

The method is specific and is capable of quantitating 5α -androst-16-en-3-one at a level as low as 50 ng/g of fat sample. The standard deviation for 27 determinations with the same sample at the lowest level of 5α -androst-16-en-3-one detectable varied by a maximum of only 4.9%. Thus, the precision and sensitivity of the method verifies the accuracy of this procedure for measuring 5α -androst-16-en-3-one and indicates that it could be used on biopsy fat samples.

Andresen (1975) has reported the development of a radioimmunoassay for 5α -androst-16-en-3-one, which is capable of measuring as little as 90 ng/g of fat from a sample of 40–60 mg of fatty tissue. The high specificity of SIM GC-MS should be useful for establishing the accuracy of radioimmunoassay, which should be faster, simpler, and lower in cost. However, the cross-reactivity of 4,16-androstadien-3-one and 5α -androst-16-en-3-one may be a shortcoming of the immunoassay, whereas, the SIM technique is extremely specific due to the uniqueness of the molecular weights coupled with the TLC and GLC behavior of the unlabeled and d_2 form of the compound.

The levels of 5α -androst-16-en-3-one found in the samples of fatty tissue are shown to vary from 0.08 to 6.97 $\mu\text{g/g}$ by the SIM GC-MS method (Table I). These values are within the range of 0.1 to 7 $\mu\text{g/g}$ reported by a number of workers for GLC analysis (Claus et al., 1971; Rhodes and Patterson, 1971; Fuchs, 1972; Stinson et al., 1972; Newell et al., 1973) of the fatty tissues from uncastrated male pigs. Andresen (1975) reported an average value of 2.09 $\mu\text{g/g}$ of fatty tissue for 5α -androst-16-en-3-one levels for intact male pigs, which compares with a mean value of 1.56 $\mu\text{g/g}$ fat in the present study for cryptorchid pigs (Table I). Unfortunately, little work has been done in correlating the levels for 5α -androst-16-en-3-one with odor scores to ascertain if the single compound is responsible.

Table I. Comparison of the Concentration of 5α -Androst-16-en-3-one and Boar Odor Intensity Scores by Packing House and Laboratory Panels

Sample no.	Concentration of 5α -androst-16-en-3-one, $\mu\text{g/g}$ of fat	Av scores for boar odor intensity	
		Packing house ^{a,c}	Lab. panel ^{b,c}
31751	0.08	1	0.3
15486	0.19	5	1.3
62342	0.22	4	3.3
63267	0.42	5	0.8
88651	0.42	4	3.3
75122	0.61	2	1.8
39528	0.67	2	1.5
17820	0.82	3	5.5
08899	1.12	3	0.5
00556	1.23	3	2.0
43253	1.24	5	1.3
94015	1.45	6	8.5
14924	1.56	4	4.8
70312	1.88	2	1.8
70707	1.88	5	3.0
02985	5.70	4	6.0
00911	6.97	5	4.8
Mean	1.56	3.70	2.97

^a Odor intensity scores for packing house panel: 0 = none, 1 = very slight, 2 = slight, 3 = moderate, 4 = slightly pronounced, 5 = pronounced, 6 = very pronounced.

^b Odor intensity scores for the laboratory panel were based on a 10-point scoring system ranging from 0 = no sex odor to 9 = very pronounced. ^c The two scoring systems differed in that the packing house panel used a consensus score in descriptive terms, thus ending up in whole numbers only. The laboratory panel values, on the other hand, are an average score from the three panel members and are expressed to the nearest 0.1. Although we were able to carefully monitor the laboratory panel, absolute control of the packing house panel was not possible.

Correlation between Panel Scores for Sex Odor and the Level of 5α -Androst-16-en-3-one. The results for chemical analysis of 5α -androst-16-en-3-one and sex odor scores for 17 cryptorchid pigs are shown in Table I. Examination of the data indicates that the odor scores and the levels of 5α -androst-16-en-3-one were not closely related for either panel. This was verified by the low correlations between sensory odor scores and the chemically determined levels of 5α -androst-16-en-3-one, which amounted to only 0.27 for the packing house panel and 0.46 for the laboratory panel. The former value was not statistically significant ($P > 0.05$), but the latter value was significant at the 5% level. These correlation coefficients agree closely with an "r" value of 0.53 between sex odor scores for pork and the level of 5α -androst-16-en-3-one as reported by Newell et al. (1973) and values of 0.51 and 0.73 obtained by Malmfors and Andresen (1975) and by Fuchs (1972), respectively. Even though the correlation coefficient between odor scores and the levels of 5α -androst-16-en-3-one for the laboratory panel in the present study was statistically significant, variability in the level of 5α -androst-16-en-3-one only accounted for 21% of the variation in panel odor scores.

The agreement between the two panels for scoring intensity of sex odor was not good with an "r" value of only 0.50. Although statistically significant at the 5% level, the variability in sensory odor scores accounted for only 25% of the variation between panels. This suggests that other factors may play an important role in sex odor scores.

The "urine-like" and "perspiration-like" nature of sex odor appears to result from a complex interplay of several undesirable odors, with 5α -androst-16-en-3-one being an

important contributor. As can be seen in Table I, a high concentration of this steroid is a good indication that the undesirable odor will be evident when the fat sample is heated. However, a low level of 5 α -androst-16-en-3-one does not guarantee freedom from undesirable odors. Therefore, it is unlikely that a chemical assay for 5 α -androst-16-en-3-one alone would be helpful as an objective test for boar odor, but an assay that included other C₁₉- Δ ¹⁶ steroids, especially the highly odoriferous 5 α -androst-16-en-3 α -ol, might give a better indication of the extent of odor to be expected in the sample. In connection with this, we noticed during the evaluation of samples in this laboratory that the distinctive musky odor of 5 α -androst-16-en-3 α -ol was noticeable in several of the samples, especially sample no. 88651 and no. 14924 (Table I).

The failure of the meat plant personnel to discriminate between samples with various levels of 5 α -androst-16-en-3-one does not discredit the hot iron technique as a way of selecting cryptorchid animals with no boar odor. All animals with levels of 5 α -androst-16-en-3-one greater than 0.1 μ g/g of fat were rated as having at least slight boar odor, which would disqualify them for use in the fresh pork market. However, the selection of carcasses eligible for use in cooked, comminuted products (less than pronounced odor), as against carcasses to be condemned or for rendering (pronounced odor), may be a more difficult task based on the observations presented here.

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National Institutes of Health, Bethesda, Md. 20014.

LITERATURE CITED

- Andresen, O., *Acta Endocrinol. (Copenhagen)* **79**, 619 (1975).
 Beery, K. E., Sink, J. D., Patton, S., Zeigler, J. H., *J. Food Sci.* **36**, 1086 (1971).
 Bishop, M. W. H., "Meat Production from Entire Male Animals", Rhodes, D. N., Ed., J. & A. Churchill, London, 1969, p 297.
 Bunnell, R. H., "The Vitamins", Vol. 6, Gyorgy, P., Pearson, W. N., Ed., Academic Press, New York, N.Y., 1967, p 97.
 Claus, K., Hoffman, B., Karg, H., *J. Anim. Sci.* **33**, 1293 (1971).
 Craig, H. B., Pearson, A. M., Webb, N. B., *J. Food Sci.* **27**, 29 (1962).
 Fuchs, G., *Swed. J. Agric. Res.* **1**, 233 (1972).
 Gaffney, T. E., Hammar, C.-G., Holmstedt, B., McMahon, R. E., *Anal. Chem.* **43**, 307 (1971).
 Gordon, A. E., Frigerio, A., *J. Chromatogr.* **73**, 401 (1972).
 Griffiths, N. M., Patterson, R. L. S., *J. Sci. Food Agric.* **21**, 4 (1970).
 Hammar, C. -G., Holmstedt, B., Ryhage, R., *Anal. Biochem.*, **25**, 532 (1968).
 Holland, J. F., Sweeley, C. C., Thrush, R. E., Teets, R. E., Bieber, M. A., *Anal. Chem.* **45**, 308 (1973).
 Kloek, J., *Psychiatr., Neurol., Neurochir.* **64**, 309 (1961).
 Malmfors, B., Andresen, O., *Acta Agric. Scand.* **25**, 92 (1975).
 Newell, J. A., Tucker, L. H., Stinson, G. C., Bowland, V. P., *Can. J. Anim. Sci.* **53**, 205 (1973).
 Patterson, R. L. S., *J. Sci. Food Agric.* **19**, 37 (1968).
 Rhodes, D. N., Patterson, R. L. S., *J. Sci. Food Agric.* **22**, 320 (1971).
 Samuelsson, B., Hamberg, M., Sweeley, C. C., *Anal. Biochem.* **38**, 301 (1970).
 Sink, J. D., *J. Theoret. Biol.* **17**, 174 (1967).
 Snedecor, G. W., "Statistical Methods", 5th ed, Iowa State University Press, Ames, Iowa, 1956, pp 160-193.
 Stinson, C. G., Tucker, L. H., Weiss, G. M., Martin, A. M., Proceedings of the 18th European Meats Research Workers Conference, 1972 p 253.
 Sweeley, C. C., Elliott, M. H., Fries, I., Ryhage, R., *Anal. Chem.* **38**, 1549 (1966).
 Sweeley, C. C., Ray, B. D., Wood, W. I., Holland, J. F., Krichevsky, M. I., *Anal. Chem.* **42**, 1505 (1970).
 Sweeley, C. C., Gates, S. C., Thompson, R. H., Harten, J., Dendramis, N., Holland, J. F., "Quant. Mass Spectrometry in Life Sciences", Elsevier, Amsterdam, 1977, p 29.
 Thompson, R. H., Jr., Pearson, A. M., Banks, K. A., *J. Agric. Food Chem.* **20**, 185 (1972).
 U.S. Department of Agriculture, "Meat and Poultry Inspection Program", M-311.20 (1973).
 Vandenheuvel, F. A., *J. Lab. Clin. Med.* **69**, 343 (1967).
 Williams, L. D., Pearson, A. M., Webb, N. B., *J. Anim. Sci.* **22**, 166 (1963).

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