

Gas Chromatographic–Mass Spectrometric Analysis of Steroids and Steroid Glucuronides in the Seminal Vesicle Fluid of the African Catfish, *Clarias gariepinus*

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Gas chromatographic–mass spectrometric analysis was carried out to identify steroids and steroid glucuronides in the seminal vesicle fluid of African catfish, *Clarias gariepinus*, collected in the Hula nature reserve (Israel) during the breeding season. Full mass spectra of 5 β -pregnane-3 α ,17 α -diol-20-one and cholesterol were obtained. After treatment with β -glucuronidase the following steroid glucuronides were determined by full mass spectra of the corresponding free steroids: etiocholanolone, 5 β -androstane-3 α ,17 β -diol-11-one, 5 β -pregnane-3 α ,17 α -diol-20-one, and cholesterol. Furthermore, after selected ion monitoring the following steroids and steroid glucuronides could be detected by the presence of at least two characteristic ions at the expected retention time: 5 β -androstane-3 β ,17 β -diol, 5 β -androstane-3 α ,17 β -diol, etiocholanolone, 5 β -androstane-3 α ,17 β -diol-11-one, testosterone, 5 β -androstane-3 α ,17 β -diol-glucuronide, and testosterone–glucuronide. These results agree with the hypothesis that steroid glucuronides, synthesized by the seminal vesicles, are excreted with the seminal vesicle fluid into the external environment, where they might function as sex pheromones. © 1987 Academic Press, Inc.

In teleosts pheromones of gonadal origin may act as sex attractants and may evoke reproductive behavior (reviews: Liley, 1982; Stacey, 1983). Apart from proteins, steroids, and cholesterol esters, steroid glucuronides have been suggested to be sex pheromones in this group of vertebrates (Colombo *et al.*, 1982; Liley and Stacey, 1983; Lambert *et al.*, 1986). In *Gobius jozo* etiocholanolone glucuronide, synthesized in the mesorchial gland, attracts gravid females (Colombo *et al.*, 1977, 1980). The same compound also attracts male goldfish, *Carassius auratus*, and guppies, *Poecilia reticulata* (Colombo *et al.*, 1982), while a mixture of testosterone–glucuronide and estradiol–glucuronide is capable of attracting male zebrafish, *Brachydanio rerio* (Van den Hurk and Lambert 1983). Furthermore, in zebrafish a testicular fraction, containing steroid glucuronides, can

induce ovulation in female conspecifics (Van den Hurk *et al.*, 1987a, b). Also for African catfish, *Clarias gariepinus*, evidence is increasing that male pheromones, in particular steroid glucuronides synthesized by the seminal vesicles, are involved in reproductive behavior by attracting females after ovulation (Lambert *et al.*, 1986; Resink *et al.*, 1987b). The results of *in vitro* incubation studies indicate that the seminal vesicle is capable of synthesizing various steroids and at least six different steroid glucuronides (Fig. 9) (Schoonen and Lambert, 1986; Schoonen *et al.*, 1987a, b; Resink *et al.*, 1987a). These steroid glucuronides are testosterone-, 5 β -androstane-17 β -ol-3-one-(5 β -dihydrotestosterone), 5 β -androstane-3 α ,17 β -diol-, 5 β -androstane-3 β ,17 β -diol-, 5 β -androstane-3 α -ol-17-one- (etiocholanolone), and 5 β -pregnane-3 α ,17 α -diol-20-one-glucuronide. In order to have a pheromonal function these compounds should be excreted with the fluid of

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the seminal vesicles into the water. Therefore, as part of our studies of male sex pheromones in the African catfish, it seemed necessary to trace the presence of these steroid glucuronides in the seminal vesicle fluid. This was done by means of gas chromatography-mass spectrometry. In addition, the seminal vesicle fluid was analyzed for the presence of 5 β -androsterane-3 α ,17 β -diol-11-one and 5 β -androsterane-3 α ,11 β -diol-17-one-glucuronide, as the free steroids of these two compounds were among the products synthesized by seminal vesicles under *in vitro* conditions (Schoonen *et al.*, 1987b). Finally, the presence of the free steroids of the above-mentioned steroid glucuronides, and of cholesterol and cholesterol-glucuronide, was investigated.

MATERIALS AND METHODS

Materials. All chemicals and solvents were of analytical grade and the solvents (Baker) were distilled twice before use. Reference steroids were obtained from Steraloids and Makor, β -glucuronidase of *Escherichia coli* (100 U/ml) from Boehringer, Sep Pak C₁₈ minicolumns from Waters Associates, and the derivatization reagents trimethylchlorosilane (TMCS) and *N,O*-bis-(trimethylsilyl)-acetamide (BSA) from Fluka.

Animals. Male African catfish, *C. gariepinus*, were collected in the Hula nature reserve, 30 km north of Lake Kinneret (Israel), during the breeding period, i.e., in June, 1984. The testes of these males contained running ripe sperm as fertilization tests confirmed. The animals were transported to the laboratory (Kinneret Limnological Laboratory, Tabgha, Israel) and killed after being anesthetized with phenoxethanol. The seminal vesicles were removed, cleaned from blood residues, cut into small pieces, and then centrifuged at 3000 rpm for 10 min. The supernatant, i.e., the seminal vesicle fluid, was collected.

Extraction. Free steroids and steroid conjugates were extracted from the seminal vesicle fluid by reversed-phase chromatography with Sep Pak C₁₈ columns. These columns were activated with methanol (2 \times 2 ml) and equilibrated with distilled water (2 \times 5 ml). After this pretreatment 2 ml of seminal vesicle fluid, diluted with distilled water to a volume of 10 ml, was transferred to the column. After the column was rinsed with 10 ml distilled water to remove remaining proteins, the steroids and steroid conjugates were eluted together with ethanol (4 \times 2 ml) and eth-

anol-distilled water (1:1) (4 \times 2 ml). The eluate was evaporated under a stream of nitrogen and the residue was redissolved in distilled water (5 ml) to dissolve the steroids and steroid conjugates. Thereafter the free steroids were extracted with dichloromethane (3 \times 10 ml) and this free steroid fraction was set apart until derivatization. In order to make sure that all free steroids had been removed, the remaining water fraction was then washed with dichloromethane (2 \times 10 ml), and subsequently evaporated. The residue of the water fraction was redissolved in 2 ml sodium acetate buffer (0.1 M, pH 6.5) and treated with 100 μ l β -glucuronidase at 37° overnight under continuous shaking in an atmosphere of air. The enzyme reaction was terminated by adding 10 ml dichloromethane, whereafter extraction of deglucuronidated steroids was carried out with dichloromethane (3 \times 10 ml).

Derivatization. Trimethylsilyl (TMS) and oxime-trimethylsilyl derivatives were prepared. The dichloromethane fractions with free and deglucuronidated steroids were put into reaction vials and evaporated under a stream of nitrogen, whereafter 200 μ l 2% hydroxylammonium chloride in pyridine was added. These mixtures were incubated for 1 hr at 100°. Steroids possessing keto groups were converted to oxime derivatives. Following evaporation 100 μ l of a freshly prepared mixture of BSA and TMCS (9:1) was added to the residue and incubated for 1 hr at 70° to obtain the TMS ether derivatives of the steroids. After evaporation, the residue was dissolved in 2 ml hexane and the polar compounds (nonsteroid derivatives) were removed by extraction with acetonitrile (2 \times 0.2 ml). Finally, the steroid derivative fraction was dissolved in 50 μ l of hexane and an aliquot of 2 μ l was subjected to GC-MS.

Capillary gas chromatography-mass spectrometry. A Hewlett-Packard 5992 B gas chromatograph-mass spectrometer with a Hewlett-Packard fused silica capillary column (ultra 1, crosslinked methyl silicone, film thickness, 0.17 μ m, 25 m \times 0.31 mm i.d.) was used with helium as carrier gas at a flow rate of 2 ml/min. The injection port temperature was 250° and the oven temperature was set at 160° and increased 1 min after injection at a rate of 15°/min to 190°, followed after 0.5 min by a second increase at a rate of 2°/min to 235°. For total ion monitoring with a scan reach of 200–600 *m/z*, the multiplier detector was set at 1800 V and for selected ion monitoring (SIM) at 2600 V. The mass spectrometer was optimized for the mid range area (*m/z* 414) and the obtained mass spectra were nonnormalized spectra.

Spectra comparison-similarity index. The spectra of both the steroids derived from the seminal vesicle fluid and the reference steroids were reduced to 10 peaks, selected on the base of the highest mass times the abundance values. From the reference steroids these 10 particular peaks were put into the library. The

algorithm used in comparing the reduced spectra of the unknowns from the seminal vesicle fluid with those of the reference steroids in the library was based on the following correlation index or similarity index equation given as a standard program in the HP-GC-MS:

$$S.I. = \frac{\sum_{m=1}^k A_m \cdot a_m}{\sqrt{\sum_{m=1}^k A_m^2 \cdot \sum_{m=1}^k a_m^2}}$$

where S.I. = similarity index ($0 \leq S.I. \leq 1$) (0 = totally different; 1 = identical), A_m = abundance of the ion at mass m in unknown spectrum, a_m = abundance of the ion at mass m in library spectrum, and k = total number of different ions ($10 \leq k \leq 20$). S.I. > 0.8 was used as an arbitrary value for a good correlation.

RESULTS

Separation of Steroids by Gas Chromatography

The results of a standard gas chromatographic run with a mixture of equal amounts (100 ng) of the steroid derivatives are shown in Fig. 1. The method employed led to a sufficient separation of the compounds. Testosterone-oxime-diTMS and 5 β -dihydrotestosterone-oxime-diTMS manifest themselves in a so-called *cis* and *trans* configuration, which results in two peaks of each steroid. The *cis* configuration of testosterone-oxime-diTMS cochromato-

graphed with the derivative of 5 β -androstande-3 α ,11 β -diol-17-one. This did not interfere with the identification of these steroids as their derivatives each have their own characteristic mass fragments. The derivative of 5 β -androstande-3 α ,17 β -diol-11-one also showed two peaks, with retention times of 21.8 and 23.1 min, respectively. This indicates the presence of two different derivatives, i.e., 5 β -androstande-3 α ,17 β -diol-11-one-diTMS with a MW of 450.2 and 5 β -androstande-3 α ,17 β -diol-11-one-oxime-triTMS with a MW of 537.4. This phenomenon influenced the detection level of the compound. Furthermore cholesterol-TMS was present in this standard chromatographic run at a retention time of 38.5 min (not shown).

Identification by GC-MS

5 β -Androstane-3 β ,17 β -diol. The TMS derivative of standard 5 β -androstande-3 β ,17 β -diol had a retention time of 17.9 min. The mass spectrum was characterized by the molecular ion with m/z 436.3 (M^+), and the mass fragment ions with m/z 421.3 ($M^+ - CH_3$), m/z 346.3 ($M^+ - OTMS$), and m/z 256.0 ($M^+ - 2 \times OTMS$) (Fig. 2A, part I). The relative abundance for the

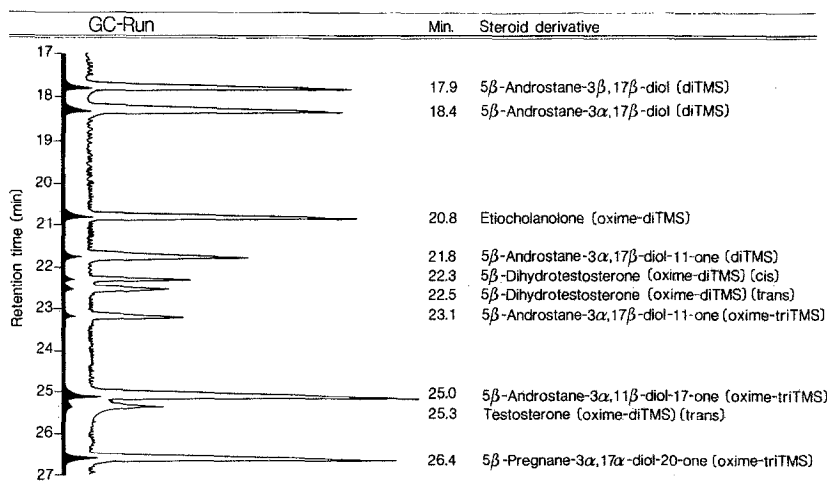


FIG. 1. Capillary gas chromatogram of derivatives of standard steroids (100 ng).

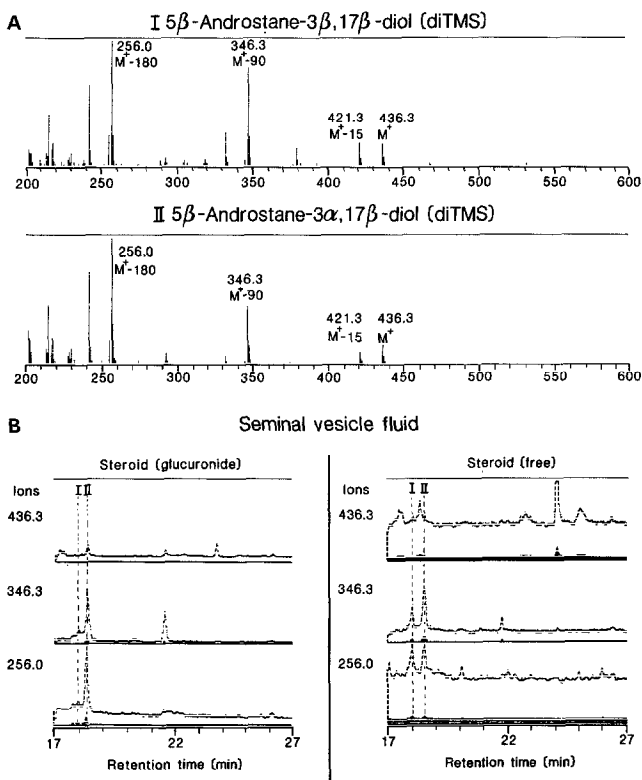


FIG. 2. (A) Mass spectra (nonnormalized) of 5 β -androstane-3 β ,17 β -diol-diTMS (I) and 5 β -androstane-3 α ,17 β -diol-diTMS (II) standards, with, as characteristic ions, the molecular ion m/z 436.3 and mass fragments m/z 421.3, m/z 346.3, and m/z 256.0. (B) SIM analysis of the derivatized steroid and steroid glucuronide fractions of the seminal vesicle fluid of *C. gariepinus* at between 17 and 27 min of the GC run. The characteristic ions of 5 β -androstane-3 β ,17 β -diol-diTMS and 5 β -androstane-3 α ,17 β -diol-diTMS were present at retention times of, respectively, 17.9 and 18.4 min in the free steroid fraction, and at a retention time of 18.4 min in the steroid glucuronide fraction.

ions 256.0, 346.3, and 436.3 was 100:79:18. It was not possible to find a full spectrum of this compound in the derivatized extracts of seminal vesicle fluid. SIM analysis, however, revealed that two of the most characteristic ions, i.e., m/z 256.0 and m/z 346.3, were present only in the free steroid fraction (Fig. 2B) at a retention time of 17.9 min with a relative abundance ratio of 100:72.

5 β -Androstane-3 α ,17 β -diol. The standard 5 β -androstane-3 α ,17 β -diol-diTMS with a retention time of 18.4 min showed the same ions as 5 β -androstane-3 β ,17 β -diol-diTMS, i.e., m/z 436.3, m/z 421.3, m/z 346.3, and m/z 256.0 (Fig. 2A, Part II), with a relative abundance ratio of 100:48:15 for

the ions 256.0, 346.3, and 436.3, respectively. Total ion monitoring of the derivatized seminal vesicle fluid fractions did not result in a full spectrum. On the other hand, SIM analysis demonstrated that the fragment ions with m/z 256.0, m/z 346.3, and even the molecular ion with m/z 436.3 were present at the retention time of 18.4 min in the steroid glucuronide fraction (Fig. 2B) with a relative abundance ratio of 100:59:14. Only an indication of the presence of 5 β -androstane-3 α ,17 β -diol-diTMS as free steroid could be obtained.

Etiocholanolone. The retention time of standard etiocholanolone-oxime-diTMS was 20.8 min. Its characteristic ions were the molecular ion with m/z 449.2 (M^+) and

the mass fragments with m/z 434.2 ($M^+ - CH_3$), m/z 360.0 ($M^+ - OTMS$), and m/z 270.0 ($M^+ - 2 \times OTMS$) (Fig. 3A). The relative abundance for the ions 270.0, 360.0, and 449.2 was 100:60:11. From the steroid glucuronide fraction a full spectrum was obtained at 20.8 min (Fig. 3B), which clearly demonstrated the presence of etiocholanolone-oxime-diTMS, since this spectrum correlated very well with the mass spectrum of the standard (S.I., 0.993). The SIM runs of the ions with m/z 449.2, m/z 360.0, and m/z 270.0 demonstrated that in addition to the steroid glucuronide fraction the free steroid fraction also contained etiocholanolone (Fig. 3C). The relative abundance for the ions 270.0, 360.0, and

449.2 was 100:70:12 for the glucuronide fraction and 100:67:12 for the free steroid fraction.

5 β -Androstane-3 α ,17 β -diol-11-one. After derivatization two different derivatives were formed: 5 β -androstane-3 α ,17 β -diol-11-one-diTMS with a retention time of 21.8 min and a mass spectrum with a molecular ion with m/z 450.2 (M^+) and typical mass fragments with m/z 435.2 ($M^+ - CH_3$), m/z 360.0 ($M^+ - OTMS$), m/z 345.2 ($M^+ - (OTMS + CH_3)$), and m/z 306.1 ($M^+ - 2 \times TMS$) (Fig. 4A). The relative abundance of ions 360.0, 306.1, and 450.2 is 100:90:26 and the theoretical abundance ratio for the ions 450.2 (M^+) and 451.2 ($M^+ + 1$) was 100:38. The second derivative, 5 β -andro-

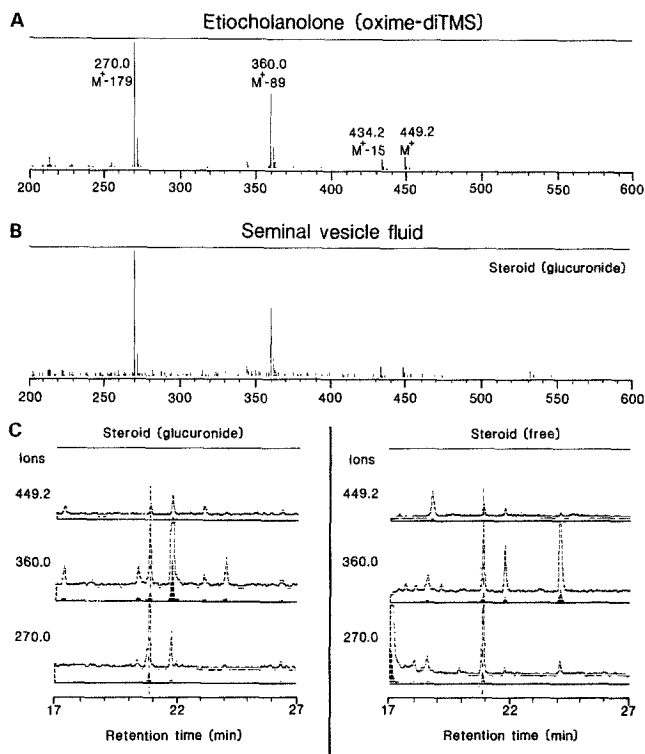


FIG. 3. (A) Mass spectrum (nonnormalized) of etiocholanolone-oxime-diTMS standard, with, as characteristic ions, the molecular ion m/z 449.2 and mass fragments m/z 434.2, m/z 360.0, and m/z 270.0. (B) Mass spectrum (nonnormalized) of the derivatized steroid glucuronide fraction of the seminal vesicle fluid of *C. gariepinus* at the expected retention time of etiocholanolone-oxime-diTMS. (C) SIM analysis of the derivatized steroid and steroid glucuronide fractions of the seminal vesicle fluid of *C. gariepinus* at between 17 and 27 min of the GC run. The characteristic ions of etiocholanolone-oxime-diTMS were present at a retention time of 20.8 min in both fractions.

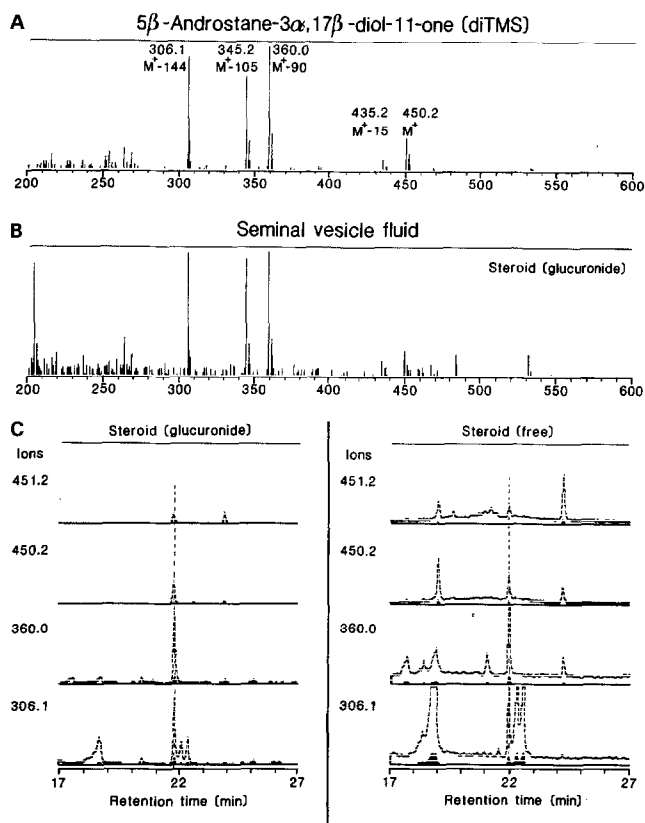


FIG. 4. (A) Mass spectrum (nonnormalized) of 5β-androstane-3α,17β-diol-11-one-diTMS standard, with, as characteristic ions, the molecular ion m/z 450.2 and mass fragments m/z 435.2, m/z 360.0, m/z 345.2, and m/z 306.1. (B) Mass spectrum (nonnormalized) of the derivatized steroid glucuronide fraction of the seminal vesicle fluid of *C. gariepinus* at the expected retention time of 5β-androstane-3α,17β-diol-11-one-diTMS. (C) SIM analysis of the derivatized steroid and steroid glucuronide fractions of the seminal vesicle fluid of *C. gariepinus* at between 17 and 27 min of the GC run. The characteristic ions of 5β-androstane-3α,17β-diol-11-one-diTMS were present at a retention time of 21.8 min in both fractions.

stane-3α,17β-diol-11-one-oxime-triTMS, had a retention time of 23.2 min and a mass spectrum with the following typical ions: a molecular ion with m/z 537.4 (M^+) and mass fragments with m/z 522.4 ($M^+ - CH_3$), m/z 446.3 ($M^+ - OTMS$), m/z 432.3 ($M^+ - (OTMS + CH_3)$), and m/z 358.2 ($M - 2 \times OTMS$) (Fig. 5A). The relative abundance ratio for the ions 446.3, 358.2, and 537.4 was 100:81:87 and the theoretical abundance ratio for M^+ (537.4), $M^+ + 1$ (538.4), and $M^+ + 2$ (539.4) is 100:47:21.

Both derivatives could be identified in the derivatized glucuronide fraction of the

seminal vesicle fluid by spectral analysis (Fig. 4B and 5B). The spectra correlated very well with the standard spectra (S.I., 0.888 and 0.848).

With SIM analysis the characteristic fragment ions of 5β-androstane-3α,17β-diol-11-one-diTMS, i.e., m/z 360.0, m/z 306.1, and m/z 450.2, could be detected in both seminal vesicle fluid fractions at a retention time of 21.8 min (Fig. 4C) and with abundance ratios of 100:89:29 for the glucuronide fraction and 100:90:29 for the free steroid fraction. The relative abundance ratios of the isotope peaks M and $M + 1$

were almost identical to the standard, i.e., 100:39 for the glucuronide fraction and 100:42 for the free steroid fraction (Fig. 4C).

The typical fragment ions of the second derivative, 5 β -androstane-3 α ,17 β -diol-11-one-oxime-triTMS, i.e., m/z 446.3, m/z 358.2, and m/z 537.4 could also be detected in both fractions at a retention time of 23.3 min with relative abundance ratios of 100:68:88 and 100:80:88 (Fig. 5C). The isotope peaks 537.4 (M^+), 538.4 ($M^+ + 1$),

and 539.4 ($M^+ + 2$) showed an abundance ratio almost identical to that of the standard. The ratio was 100:49:22 in the glucuronide fraction and 100:48:21 in the free steroid fraction.

5 β -Dihydrotestosterone. The oxime-diTMS derivatives of 5 β -dihydrotestosterone, the *cis* and *trans* configuration, had retention times of 22.3 and 22.5 min, respectively, and their mass spectra showed the same characteristic ions as etiocholanolone-oxime-diTMS, i.e., m/z 449.2, m/z

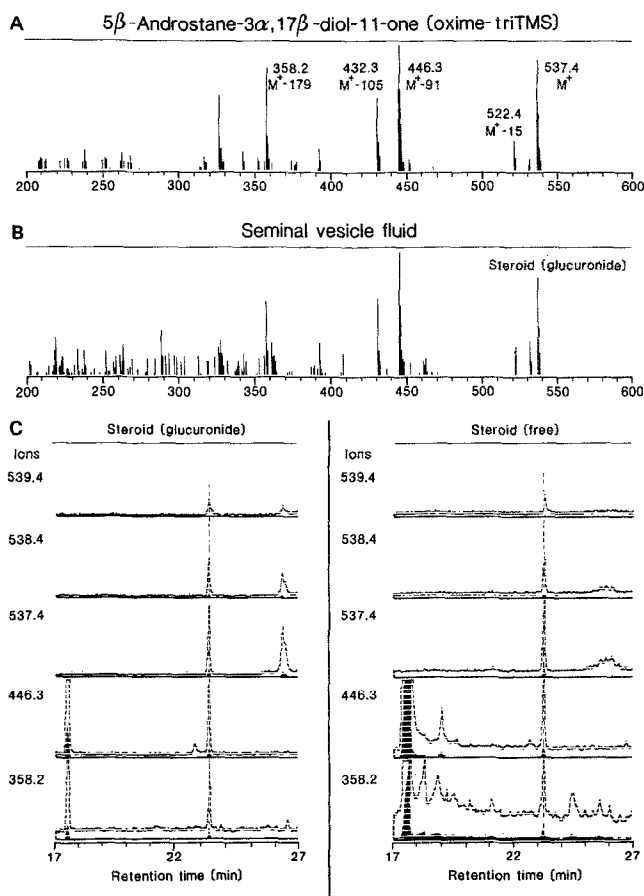


FIG. 5. (A) Mass spectrum (nonnormalized) of 5 β -androstane-3 α ,17 β -diol-11-one-oxime-triTMS standard, with, as characteristic ions, the molecular ion m/z 537.4 and mass fragments m/z 522.4, m/z 446.3, m/z 432.3, and m/z 358.2. (B) Mass spectrum (nonnormalized) of the derivatized steroid glucuronide fraction of the seminal vesicle fluid of *C. gariepinus* at the expected retention time of 5 β -androstane-3 α ,17 β -diol-11-one-oxime-triTMS. (C) SIM analysis of the derivatized steroid and steroid glucuronide fraction of the seminal vesicle fluid of *C. gariepinus* at between 17 and 27 min of the GC run. The characteristic ions of 5 β -androstane-3 α ,17 β -diol-11-one-oxime-triTMS were present at a retention time of 23.1 min in both fractions.

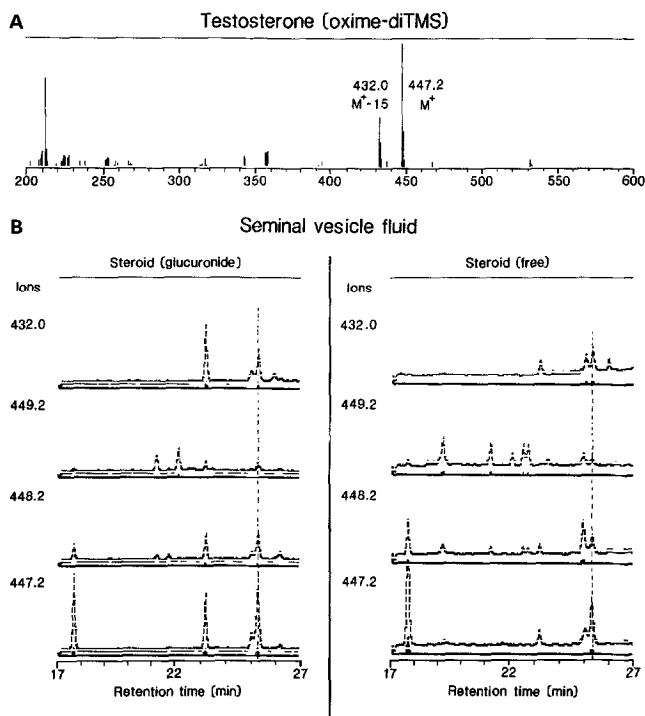


FIG. 6. (A) Mass spectrum (nonnormalized) of testosterone-oxime-diTMS standard, with, as characteristic ions, the molecular ion m/z 447.2 and mass fragment m/z 432.0. (B) SIM analysis of the derivatized steroid and steroid glucuronide fractions of the seminal vesicle fluid of *C. gariepinus* at between 17 and 27 min of the GC run. The characteristic ions of testosterone-oxime-diTMS were present at a retention time of 25.3 min in both fractions.

360.0, and m/z 270.0. None of these ions could be identified at retention times of 22.3 and 22.5 min (Fig. 3C).

5 β -Androstane-3 α ,11 β -diol-17-one. The oxime-triTMS derivative of this steroid had a retention time of 25.0 min and its characteristic ions were a molecular ion with m/z 537.4 (M^+) and the mass fragments with m/z 448.2 ($M^+ - OTMS$) and 267.8 ($M^+ - 3 \times OTMS$). It was not possible to demonstrate this steroid. Even SIM analysis did not reveal the identification of this compound. A SIM run of one of the characteristic ions (m/z 448.2) is shown in Fig. 6B.

Testosterone. Only the *trans* configuration of testosterone-oxime-diTMS with a retention time of 25.3 min was used for identification. The spectrum of the standard showed two characteristic ions, i.e., the molecular ion (M^+) with m/z 447.2 and

its fragment ($M^+ - CH_3$) with m/z 432.0. The relative abundance ratio of these ions was 100:41 (Fig. 6A), and the theoretical ratio of the molecular isotopes m/z 447.2 (M^+), m/z 448.2 ($M^+ + 1$), and m/z 449.2 ($M^+ + 2$) was 100:38:14. After analysis of the steroid glucuronide and the free steroid fractions, it appeared that in both fractions testosterone could be identified only with SIM analysis. The SIM runs of the four ions mentioned above are presented in Fig. 6B.

From these runs the abundance ratios of M^+ and $M^+ - CH_3$ (100:42) and of M^+ , $M^+ + 1$, and $M^+ + 2$ (100:39:14) for the steroid glucuronide fraction and of M^+ and $M^+ - CH_3$ (100:43) and of M^+ , $M^+ + 1$, and $M^+ + 2$ (100:39:17) for the free steroid fraction were calculated.

5 β -Pregnane-3 α ,17 α -diol-20-one. The

mass spectrum of the oxime-triTMS derivative of 5β -pregnane- $3\alpha,17\alpha$ -diol-20-one was characterized by the molecular ion with m/z 565.3 (M^+) and the mass fragments with m/z 550.3 ($M^+ - CH_3$), m/z 476.3 ($M - OTMS$), m/z 422.2 ($M^+ - 2 \times TMS$), and m/z 246.1 ($M^+ - 319$) (Fig. 7A). The retention time of this compound was 26.4 min. The relative abundance of the ions 422.2, 246.1, 476.3, and 565.3 was 100:73:59:11. The same spectrum could be detected in the glucuronide fraction and in

the free steroid fraction (Fig. 7B) of the seminal vesicle fluid with a similarity index of 0.999 and 0.861, respectively, and with a retention time of 26.4 min. In the glucuronide fraction as well as in the free steroid fraction SIM of the four most important ions 565.3, 476.3, 422.2, and 246.1 showed sharp intensive peaks for all these ions at a retention time of 26.4 min (Fig. 7C) with relative abundances of 100:51:71:13 and 100:52:69:12, respectively.

Cholesterol. The presence of chole-

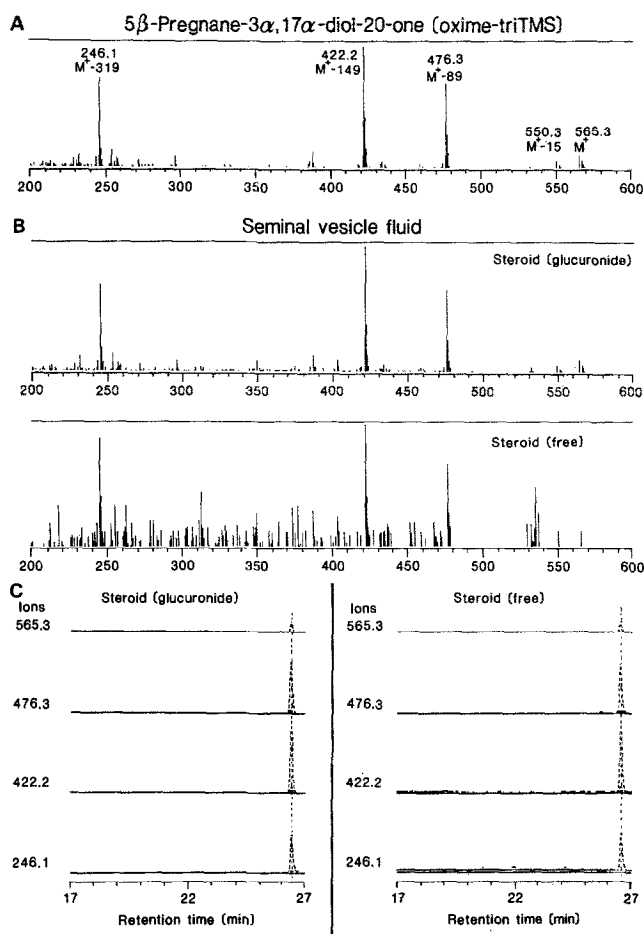


FIG. 7. (A) Mass spectrum (nonnormalized) of 5β -pregnane- $3\alpha,17\alpha$ -diol-20-one-oxime-triTMS standard, with, as characteristic ions, the molecular ion m/z 565.3 and mass fragments m/z 476.3, m/z 422.2, and m/z 246.1. (B) Mass spectrum (nonnormalized) of the derivatized steroid and steroid glucuronide fractions of the seminal vesicle fluid of *C. gariepinus* at the expected retention time of 5β -pregnane- $3\alpha,17\alpha$ -diol-20-one-oxime-triTMS. (C) SIM analysis of the derivatized steroid and steroid glucuronide fractions of the seminal vesicle fluid of *C. gariepinus* at between 17 and 27 min of the GC run. The characteristic ions of 5β -pregnane- $3\alpha,17\alpha$ -diol-20-one-oxime-triTMS were present at a retention time of 26.4 min in both fractions.

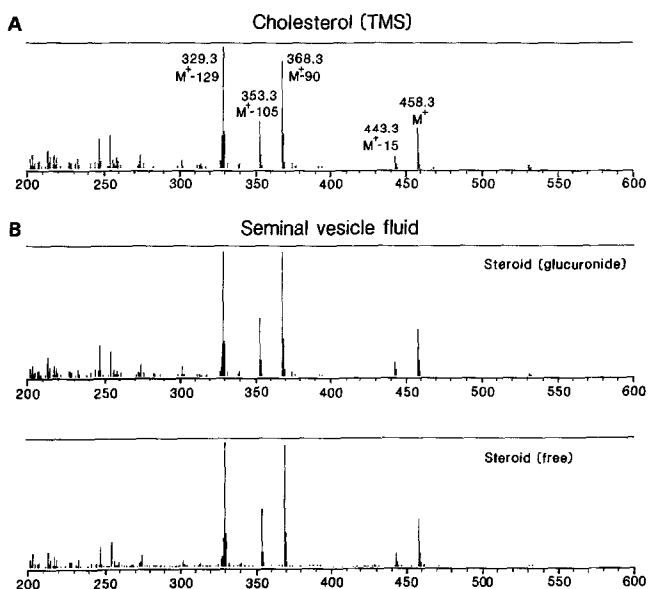


FIG. 8. (A) Mass spectrum (nonnormalized) of cholesterol-TMS, with, as characteristic ions, the molecular ion m/z 458.3 and mass fragments m/z 443.2, m/z 368.3, m/z 353.3, and m/z 329.3. (B) Mass spectrum (nonnormalized) of the derivatized steroid and steroid glucuronide fractions of the seminal vesicle fluid of *C. gariepinus* at the expected retention time of cholesterol-TMS.

terol-TMS could also be demonstrated in both the glucuronide and free steroid fraction. A full spectrum of cholesterol-TMS could be detected at a retention time of 38.5 min (Fig. 8B). The mass spectra correlated very well with those of the standard (Fig. 8A). The similarity index was 0.982 for the glucuronide fraction and 0.981 for the free steroid fraction.

DISCUSSION

Gas chromatographic-mass spectrometric analysis of seminal vesicle fluid of African catfish, *C. gariepinus*, captured during the breeding period in their natural habitats in Israel resulted in the identification of several steroids and their glucuronides. The steroids demonstrated, both as steroid glucuronides and as free steroids, were testosterone, 5β -androstane- $3\alpha,17\beta$ -diol, etiocholanolone, 5β -androstane- $3\alpha,17\beta$ -diol-11-one, and 5β -pregnane- $3\alpha,17\alpha$ -diol-20-one. Moreover cholesterol

and its glucuronide could be detected in the seminal vesicle fluid. 5β -Androstane- $3\beta,17\beta$ -diol was detectable only as free steroid, while 5β -dihydrotestosterone and 5β -androstane- $3\alpha,11\beta$ -diol-17-one could not be demonstrated at all, although these steroids were present after *in vitro* incubations of seminal vesicle tissue (Schoonen and Lambert, 1986; Schoonen *et al.*, 1987b; Resink *et al.*, 1987a).

It is possible that the failure to demonstrate 5β -dihydrotestosterone can be ascribed to the low response of this steroid during GC-MS analysis, and to the fact that its oxime-diTMS derivative is divided into the *cis* and *trans* configurations. The absence of 5β -androstane- $3\alpha,11\beta$ -diol-17-one, on the other hand, can only be explained by very low amounts of this steroid in the seminal vesicle fluid.

These results of GC-MS analysis of steroids and steroid glucuronides in the seminal vesicle fluid agree with the data of the seminal vesicle incubation studies men-

tioned in the introduction. This indicates that *in vitro* incubation studies of minced steroid-producing tissues, using radiolabeled steroids as precursors, can give a good idea not only of the enzyme systems

involved in steroidogenesis, but also of the metabolic products secreted by such a tissue (Fig. 9).

According to Resink *et al.* (1987b) seminal vesicle fluid and, in particular, its frac-

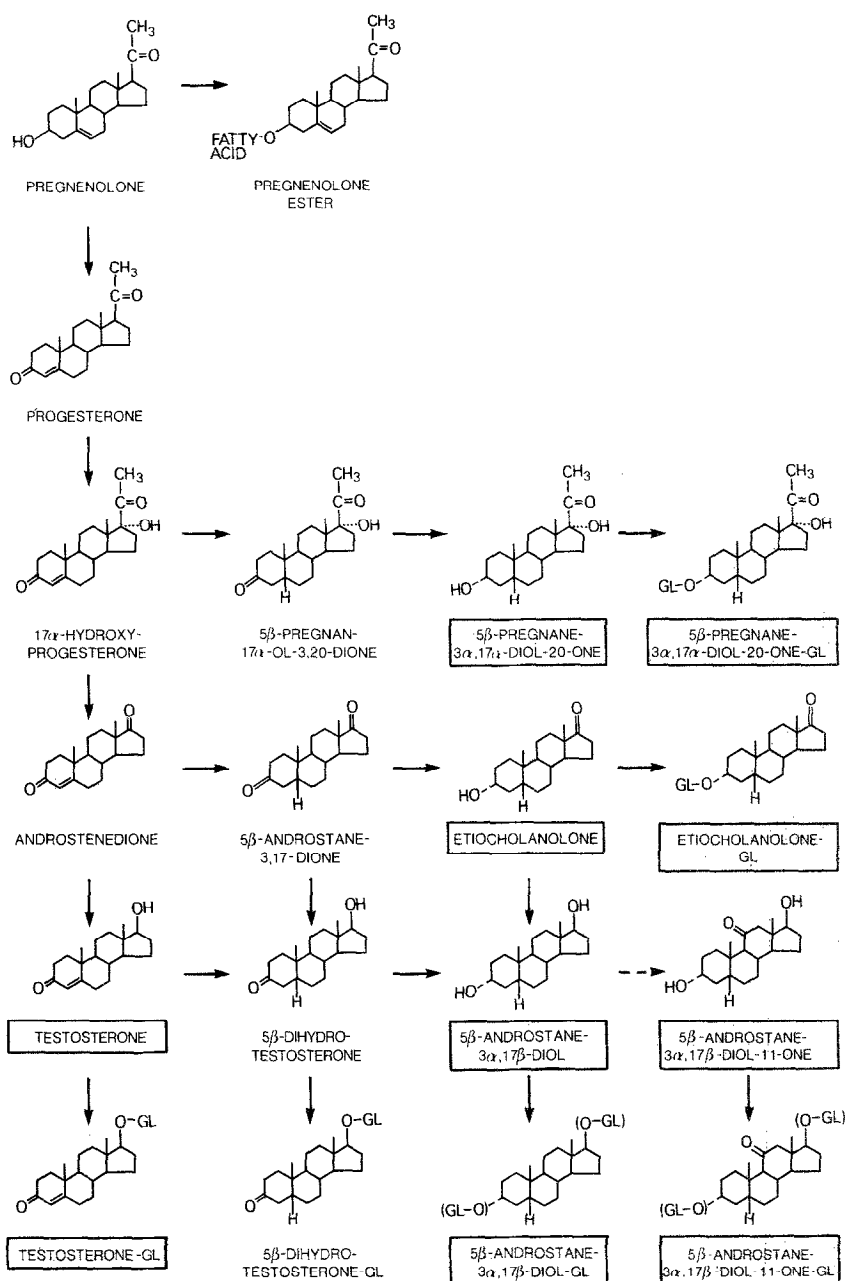


FIG. 9. Steroidogenic pathway in the seminal vesicles of the African catfish, *Clarias gariepinus*, and the identified products in seminal vesicle fluid (\square).

tions containing steroid glucuronides are involved in attracting ovulated females immediately before the onset of nuptial behavior in the African catfish. It seems possible that it is a mixture of these steroid glucuronides rather than one or two of them that acts as sex attractants in *C. gariepinus*.

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