

$\Delta^{8(14)}$ -Cholestene (300 mg.) was oxidized with chromic acid by the same procedure as used in the oxidation of  $\Delta^8$ -cholestene,<sup>2</sup> and the reaction product was divided into a ketone and a hydrocarbon fraction. The ketone fraction was indicated to contain a mixture of ketones since a crystalline product was not obtained either directly or as a semicarbazone or an oxime. The hydrocarbon fraction on repeated recrystallization from acetone-methanol yielded 12 mg. of  $\Delta^{8,14}$ -cholestadiene, m. p. 83–84°, which gave no depression in mixed melting point with a sample of  $\Delta^{8,14}$ -cholestadiene obtained by the action of perbenzoic acid on  $\Delta^{8(14)}$ -cholestene.

$\Delta^{8,14}$ -Cholestadiene was recovered unchanged after being refluxed with maleic anhydride in benzene for nine hours. Catalytic hydrogenation of  $\Delta^{8,14}$ -cholestadiene in ethyl acetate with palladium catalyst yielded a compound in the form of needles, m. p. 52–54°, which gave no depression in mixed melting point with  $\Delta^{8(14)}$ -cholestene.

**Bromine Titrations.**—The cholestadienes were titrated with bromine in the same manner as that described for the titration of  $\Delta^8$ -,  $\Delta^{8(14)}$ - and  $\Delta^{14}$ -cholestenes.<sup>2</sup> It was found that  $\Delta^{8,8}$ -cholestadiene,  $\Delta^{6,8(14)}$ -cholestadiene,

$\Delta^{7,9(11)}$ -cholestadiene,  $\Delta^{7,14}$ -cholestadiene and  $\Delta^{8,14}$ -cholestadiene in chloroform solution consumed 1.01, 2.24, 3.04, 2.66 and 3.22 molar equivalents of bromine, respectively, dissolved in chloroform. Using a methanol solution of the compound and a methanol solution of bromine, the same compounds consumed 1.02, —, 1.65, 1.76 and 1.83 molar equivalents of bromine, respectively.

The authors wish to express their appreciation to Mr. E. M. Gladrow of the physical chemistry department for helpful assistance in obtaining the absorption spectrum data.

### Summary

$\Delta^{6,8(14)}$ -,  $\Delta^{7,9(11)}$ -,  $\Delta^{7,14}$ - and  $\Delta^{8,14}$ -cholestadienes were prepared. The methods of preparation and the specific rotations of these cholestadienes were compared with those of the known analogous unsaturated steroid derivatives.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF HARVARD UNIVERSITY AND LOUISIANA STATE UNIVERSITY]

## American Musk. I. The Chemical Constitution of the Musk of the Louisiana Muskrat<sup>1</sup>

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It is now 36 years since Walbaum<sup>4</sup> discovered that the essential principle of musk was muscone, 30 years since Sack<sup>5</sup> isolated civetone from civet, and 16 years since the classic work of Ruzicka<sup>6</sup> who elucidated the structure of these curious compounds. Yet in spite of the interest shown in this field, the most abundant natural source<sup>7</sup> of the large ring compounds—the scent glands of the common muskrat—apparently has been overlooked by organic chemists, a remarkable fact, since from the name *muskrat*, one would have expected an investigation to have been made long ago.

The presence of substances with a musk odor in muskrat scent glands has, however, been long suspected, but very little attention has been given

them, although they have been reported<sup>8</sup> as being about equal to civet and musk-xylene, a very shrewd estimation as this work will reveal. The only prior chemical work in the literature is an analysis of the fatty acids of these glands.<sup>9</sup>

The scent glands investigated in this work were those of the Louisiana muskrat, *Ondatra zibethicus rivalicinus*, a typical North American muskrat. Both sexes have these glands,<sup>10</sup> just as does the beaver, and the civet cat of Abyssinia, in contrast to the musk deer of Asia, the male of which alone produces musk.

**Isolation of the Crude Musk.**—The scent glands, varying in size from 0.6 g. to 1.8 g. (average, 1.25 g.),<sup>11</sup> were obtained directly from the trapper (December–February) who merely cut them from the carcass and stored them in jars of alcohol. The glands, which had a characteristic, not unpleasant, musky odor, were drained from the

(1) Preliminary work was started at the Massachusetts Institute of Technology and Louisiana State University, and continued at McGill University. The final and successful work, however, was carried out at Harvard University and Louisiana State University.

(2) Present address: Department of Chemistry, Yale University.

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(4) Walbaum, *J. prakt. Chem.*, [2] **73**, 488 (1906).

(5) Sack, German Patent 279,313 (1912); *Chem.-Ztg.*, **39**, 538 (1915).

(6) Ruzicka, *Helv. Chim. Acta*, **9**, 280, 715 (1926).

(7) Approximately 6 million muskrats are trapped annually in Louisiana alone.

(8) Redgrove, *Chemist and Druggist*, **112**, 288 (1930).

(9) Simmons and Hills, *Analyst*, **58**, 154 (1933).

(10) The purposes of these preputial glands is not fully established, but it is said that their secretions assist the muskrat to become aware of other members of the specie, even at great distances. Any sexual significance is problematical, but this seems likely, since in both sexes the musk glands reach their maximum development in the rutting season (Grinnell, Dixon and Linsdale, "Fur Bearing Animals of California," Vol. II, University of California Press, Berkeley, California, 1937, p. 744).

(11) Dry weight about one-half this.

alcohol, washed with ether, and ground up in a meat chopper. The ground glands were extracted with ether in a Soxhlet extractor, and the extract, the preserving alcohol, the wash ether, and the expressed juices were combined and evaporated to a small volume. From 2650 fresh glands (3320 g.) there was obtained approximately 600 g. of crude extracted musk. This product was saponified with 10% potassium hydroxide in boiling alcohol, just as Ruzicka did with musk and civet.<sup>6</sup> After pouring into a large volume of water, the products were separated into acid and neutral fractions by extraction with ether. The ether extracts on evaporation gave 201 g. of neutral musk oil of a fine fragrance. Distillation of this material at 1 mm. yielded 71 g. of a light yellow oil, b. p. 130–170°, which solidified to a waxy substance with a fine musk odor. The residue yielded no more volatile products even at very low pressures. The yield of crude distilled musk was about 2.1% of the weight of the fresh glands, and 33% of the unsaponifiable substances. On redistillation at 0.3 mm. the material boiled at 122–157°.

This new musk, upon analysis,<sup>11a</sup> gave values agreeing fairly closely with  $C_{16}H_{32}O$ , and resembled several of the large ring substances obtained by Ruzicka in his study of Asiatic musk, and civet; but the new musk differed from either of these latter products in that it formed no appreciable amounts of semicarbazone, and accordingly was not ketonic in character. Furthermore it was saturated to bromine, and was only faintly optically active.<sup>12</sup> The above empirical formula and the chemical properties together with the wide boiling point range indicated that the distilled musk oil was a mixture<sup>13</sup> consisting chiefly of large ring carbinols, admixed with odoriferous ketones to give it the musk odor.<sup>14</sup> While attempts to separate the mixture failed either by conversion to the phthalates, or through the semicarbazones of the corresponding ketones (formed by oxidation of the carbinols), the mixture was separated successfully by fractional distillation<sup>15</sup> through a 60-cm. modified Podbielniak-type column having a glass spiral. From 68.9 g. of the musk oil, b. p. 122–157° (0.3 mm.), the following fractions were obtained, all of which had a more or less pronounced musk odor.

| Fraction | B. p. (1 mm.)<br>°C. | Approx.<br>amount | Remarks                               |
|----------|----------------------|-------------------|---------------------------------------|
| 1        | 128–134              | 1.5 ml.           | Yellow, solidified when chilled       |
| 2        | 134–143              | 25                | } Colorless, solidified spontaneously |
| 3        | 143–152              | 5                 |                                       |
| 4        | 152–155              | 40                | } Brownish                            |
| Residue  |                      | 2.5               |                                       |

### Carbinol and Ketone "A"

Fraction 4 was redistilled<sup>15</sup> through the same column until the boiling point reached 155° (1 mm.), and the residual higher boiling material was distilled in a modified Claisen flask, b. p. 155–157° (1 mm.), m. p. 71–74°. As this material had a weak but definite musk odor, it was treated with semicarbazide to remove traces of ketones, and the ketone-free carbinol "A" now had no musk odor (just as Ruzicka reported for muscol<sup>6</sup>), and melted somewhat higher, 73–75°,  $[\alpha]^{25}_D +0.95^\circ$  (ether).

The carbinol was shown to be essentially dihydrocivetol (cycloheptadecanol) since oxidation with chromic acid yielded a ketone "A" which proved to be dihydrocivetone. A small amount of this ketone was isolated from the ketone impurities in fraction 4 in the form of its semicarbazone (m. p. 186° alone, and 187–189° when mixed with the semicarbazone, m. p. 188.5–189.5°, of dihydrocivetone), which showed that dihydrocivetone occurs naturally in the musk oil. Although fractional distillation of the dihydrocivetone obtained by oxidation of the carbinol, followed by repeated recrystallization of its semicarbazone, failed to achieve complete purification, a comparison (Table I) of the properties of the carbinol and ketone "A," and of their transformation products, with those reported by Ruzicka, *et al.*, showed striking agreement, and the admixture of the ketone and its semicarbazone with authentic specimens of dihydrocivetone and its semicarbazone, respectively, gave no depression of the melting points.

### Carbinol and Ketone "B"

Refractionation at 1 mm. of the lower boiling material, fractions 1 and 2, yielded a small forerun (about 1 ml.), b. p. 125°, and a main fraction, b. p. 136.6–138.2°, m. p. 72–75°. The latter had a musk odor, and therefore was treated with semicarbazide. When ketone-free, carbinol "B" (m. p. 77–78°)<sup>16</sup> like carbinol "A" had no musk odor, and was shown to be essentially cyclopentadecanol (nor-muscol, or exaltol), since upon chromic acid

(16) Ruzicka, Stoll, Huyser and Boekennoogen reported 80–81° for exaltol, *Helv. Chim. Acta*, **13**, 1152 (1930).

(11a) The material used here, b. p. 139–144° (1 mm.), m. p. 59–62.5°, was obtained from an earlier extraction.

(12)  $[\alpha]^{25}_D +1.7^\circ$  (ether). After purification through the phthalate, the material had approximately the same rotation,  $[\alpha]^{25}_D +1.4^\circ$  (ether). This activity is possibly due to the presence of small amounts of asymmetric homologs of the large ring compounds identified in this musk oil, and thus may account for the different bouquet of musk odor compared with a simple mixture of the two principal ketones.

(13) This complication was not encountered by Ruzicka<sup>6</sup> with musk and civet since, in each of these cases, he found only compounds having the same size ring.

(14) Ruzicka did not report finding any muscol with muscone, but Ruzicka, Schinz and Seidel [*Helv. Chim. Acta*, **10**, 695 (1927)] found civetol with civetone.

(15) Carried out in a dry nitrogen atmosphere.

TABLE I

|                 | B. p., °C. | Mm.              | M. p., °C.         | B. p.               | DEHYDRATION PRODUCT $\xrightarrow{+2H}$ |                 | REDUCTION PRODUCT |                      |
|-----------------|------------|------------------|--------------------|---------------------|---|-----------------|-------------------|----------------------|
|                 |            |                  |                    |                     | $C_{17}H_{32}$                          |                 | $C_{17}H_{34}$    |                      |
|                 |            |                  |                    |                     | Mm.                                     | M. p., °C.      | M. p.             | $n_D^{20}$           |
| Dihydrocivetol  |            |                  |                    |                     |   |                 |                   |                      |
| This paper      | 157        | 1                | 73-75              | 119                 | 0.7                                     | 45.9-46.9       | 66.0-66.2         | 1.4538               |
| Ruzicka         | 155        | 0.5 <sup>a</sup> | 80 <sup>a</sup>    | 115                 | 0.3                                     | 47 <sup>a</sup> | 65 <sup>a</sup>   | 1.4540 <sup>b</sup>  |
| Dihydrocivetone |            |                  |                    |                     |   |                 |                   |                      |
|                 |            |                  |                    |                     |   |                 | M. p., °C.        |                      |
|                 |            |                  |                    |                     |   |                 | Oxime             | Isoxime              |
| This paper      | 189        | 11               | 59-61              | 1.4622              | 190-190.5                               |                 | 63-64             | 121.5-123            |
| Ruzicka         | 145        | 0.3 <sup>c</sup> | 63-64 <sup>c</sup> | 1.4602 <sup>b</sup> | 191 <sup>c</sup>                        |                 | 63 <sup>c</sup>   | 123-124 <sup>c</sup> |

<sup>a</sup> Ref. 14. <sup>b</sup> Ruzicka, Brugger, Pfeiffer, Schinz and Stoll, *Helv. Chim. Acta*, 9, 499 (1926). <sup>c</sup> Ref. 6.

oxidation it yielded a ketone "B," which was identified as cyclopentadecanone (nor-muscone, or exaltone) through the semicarbazone, m. p. 185-186° alone, and 186-187° when mixed with an authentic specimen, m. p. 186-187°. A small amount of this ketone<sup>17</sup> was isolated from the forerun, b. p. 125° (1 mm.), which proved that cyclopentadecanone was present in the original musk oil. The odor of the ketone was about the same as that of exaltone, but inasmuch as the ketone melted 7-10° lower than exaltone, the 2,4-dinitrophenylhydrazones and the *l*-menthydrazones were compared. In neither case was a depression of the melting point observed, and a mixture of ketone "B," m. p. 55-58°, with authentic exaltone (Akatos, Inc., New York, m. p. 63-65.5°) melted at 56-63.5°. The low melting points of our carbinols and ketones can be attributed to traces of impurities, which, due to the rather large cryoscopic constants,<sup>18</sup> depress the melting points considerably. Fortunately this property does not appear to be extended to the derivatives used for identification.

The musk of the Louisiana muskrat therefore consists of the carbinols dihydrocivetol (58%) and nor-muscol (40%) together with the ketones (2%) dihydrocivetone and nor-muscone.<sup>19</sup> The scent glands of other animals are now being investigated.

### Further Experimental Details

**Purification of the Musk Carbinols.**—Small samples of carbinols "A" and "B" were treated with semicarbazide

(17) As the semicarbazone, m. p. 186-187°, -0.6 g.

(18) Ziegler and Aurnhammer [*Ann.*, 513, 43 (1934)] have found that dihydrocivetone has a cryoscopic constant of 25, whereas civetone has the much higher value of 39. Since this high value may be due to the double bond in the ring, it might be expected that the constant for cycloheptadecene would likewise be high. A determination gave the value of 20.2, which is almost identical with that of cyclohexane, and accordingly the high value for civetone must be due to interaction of the carbonyl group and the double linkage. Conjugation through space intramolecularly would cause the molecule to become somewhat more sphere-like, and so agree with the theory of Pirsch [*Ber.*, 68, 87 (1935)].

(19) The approximate percentages apply to the distilled musk.

in aqueous methanol. The next day the products were poured into water, extracted with petroleum ether (b. p. 35-47°), the extracts washed thoroughly with water, cooled to -20° for one hour, filtered and evaporated. The residual ketone-free carbinols were distilled in high vacuum. Carbinol "A," m. p. 73-75° (ps),<sup>20</sup> *Anal.* Calcd. for  $C_{17}H_{34}O$ : C, 80.3; H, 13.5. Found: C, 80.3; H, 13.3. Carbinol "B," b. p. 123-125° (0.4 mm.), m. p. 77-78° (ps). *Anal.* Calcd. for  $C_{15}H_{30}O$ : C, 79.6; H, 13.4. Found: C, 79.8; H, 13.2.

**Oxidation of Carbinol "A."**—Oxidation of 1.5 g. was carried out as described by Ruzicka, Schinz and Seidel<sup>14</sup> with chromic acid in a benzene and water mixture with added sulfuric acid. Very little dibasic acid was formed. The product was converted to the semicarbazone, 1.5 g., which after five recrystallizations from methanol melted at 188.5-189.5° (ps). Steam distillation from oxalic acid regenerated the ketone, which, after distillation at 0.15 mm., melted at 58-61°.

**Purification of Ketone "A."**—Fourteen grams of ketone, from once recrystallized semicarbazone, was fractionally distilled<sup>15</sup> in the 60-cm. modified Podbielniak-type column. After a considerable lower-boiling forerun, the main fraction, 9.7 g., boiled at 188.5-189.5° (1 mm.). This was purified by 5 recrystallizations of the semicarbazone, m. p. 189.5-190.5° (ps), regeneration with oxalic acid, and distillation at 0.2 mm., m. p. 59-61° (ps),  $n_D^{20}$  1.4622. *Anal.* Calcd. for  $C_{17}H_{32}O$ : C, 80.9; H, 12.8. Found: C, 81.1; H, 12.9. The semicarbazone was recrystallized a sixth time, m. p. 190-190.5° (ps). *Anal.* Calcd. for  $C_{18}H_{36}ON_2$ : N, 13.6. Found: N, 13.3. The oxime, after eight recrystallizations from methanol, melted at 63-64°. *Anal.* Calcd. for  $C_{17}H_{32}ON$ : N, 5.2. Found: N, 5.1. The isoxime (cyclic amide), after seven recrystallizations from petroleum ether (b. p. 30-60°) formed plates,<sup>21</sup> melting at 121.5-123° (ps). *Anal.* Calcd. for  $C_{17}H_{32}ON$ : N, 5.2. Found: N, 5.2. The 2,4-dinitrophenylhydrazone,<sup>22</sup> orange needles from methanol, melted at 84.5-86° (ps).

**Oxidation of Carbinol "B."**—Oxidation was carried out as above for Carbinol "A," 1.55 g. of carbinol yielding 1.74 g. of semicarbazone, which, after four recrystallizations from methanol, melted at 185-186° (ps). Regeneration gave ketone "B," which, after distillation at 0.15 mm., melted at 55-58°. The 2,4-dinitrophenylhydrazone, golden-yellow needles<sup>23</sup> from ethanol, melted at 108-109°

(20) "ps" indicates preliminary softening and sintering.

(21) Prepared as described by Ruzicka (ref. 6).

(22) Prepared according to Brady, *J. Chem. Soc.*, 758 (1931).

(23) Brady (ref. 22) reported plates, m. p. 105°.

(ps). The sample from authentic exaltone melted at 106–109° (ps), and there was no depression below 106° when both specimens were mixed. The *l*-menthydrazone<sup>24</sup> formed needles from dilute ethanol, m. p. 138.5–139.5°, and when mixed with an authentic specimen had the same melting point. *Anal.* Calcd. for  $C_{26}H_{48}O_2N_2$ : N, 6.7. Found: N, 6.7.

**Dehydration of Carbinol "A."**—Five grams of carbinol was heated with 15 g. of powdered fused potassium acid sulfate for two hours at 190–200°, then one hour more at 210°. The products were worked up as usual, and distilled, b. p. 116–121° (0.7 mm.), mostly 118.5–119°. It was redistilled from sodium, b. p. 128.5–129° (1 mm.), 3.2 g. The distillate partially solidified when chilled. After laborious fractional crystallization from methanol, the needles melted at 45.9–46.9°. *Anal.* Calcd. for  $C_{17}H_{32}$ : C, 86.4; H, 13.6. Found: C, 86.4; H, 13.8.

**Reduction of Cycloheptadecene.**—The above hydrocarbon, 0.45 g., absorbed one equivalent of hydrogen rapidly in ether-acetic acid solution with Adams platinum catalyst (calcd., 66 cc. hydrogen; found, 68 cc.).<sup>25</sup> The product was freed of acid by extraction with caustic, the ethereal solution was evaporated, and the residue was recrystallized from methanol, yielding long slender needles, m. p. 66.0–66.2°,  $n_D^{25} 1.4538$ . *Anal.* Calcd. for  $C_{17}H_{34}$ : C, 85.6; H, 14.4. Found: C, 85.3; H, 14.5.

(24) Prepared according to Woodward, Kohman and Harris, *THIS JOURNAL*, **69**, 120 (1941). We are indebted to Dr. R. B. Woodward for the *l*-menthydrazone used.

(25) We are indebted to Dr. R. C. Jones for assistance in this reduction.

**Reduction of Civetone.**—Authentic civetone (0.35 g.; Firmenich and Co., New York) was hydrogenated as above, but in pure ether solution.<sup>26</sup> The correct amount of hydrogen for one double bond was absorbed rapidly, the carbonyl group remaining completely unattacked. The ether was evaporated, and the residue converted directly to the semicarbazone, which, after two recrystallizations from methanol, melted at 190–190.5° (ps). When mixed with the semicarbazone of ketone "A," no depression was observed. Regeneration with oxalic acid yielded the pure ketone, m. p. 64–65° (ps). A mixture with ketone "A," m. p. 59–61° (ps), melted at 60.5–62° (ps).

#### CRYOSCOPIC DATA

| $C_{17}H_{32}$ ,<br>mg. | Naphtha-<br>lene,<br>mg. | M. p. pure,<br>°C. | M. p. mixt.,<br>°C. | $t$ , °C. | Con-<br>stant |
|-------------------------|--------------------------|--------------------|---------------------|-----------|---------------|
| 25.762                  | 1.093                    | 46.5               | 39.7                | 6.8       | 20.5          |
| 30.173                  | 1.983                    | 46.5               | 36.3                | 10.2      | 19.9          |
| Average                 |                          |                    |                     |           | 20.2          |

#### Summary

1. The scent glands of the Louisiana muskrat contain a mixture of cyclopentadecanol and cycloheptadecanol, and the corresponding ketones.

2. The cryoscopic constant of cycloheptadecene has been determined.

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BATON ROUGE, LOUISIANA RECEIVED SEPTEMBER 20, 1941

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

## Sterols. CXXV. Sapogenins. LI. The Structure of the Dibasic Acid Obtained by the Permanganate Oxidation of Anhydrosarsasapogenoic Acid

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Our experimental work shows that the dibasic acid obtained on the oxidation of anhydrosarsasapogenoic acid by potassium permanganate cannot have the structure (III) given by Fieser and Jacobsen.<sup>1</sup> We suggest structure II for this acid.

We wish to thank Parke, Davis and Company for their assistance.

**Alkaline Permanganate Oxidation of Anhydrosarsasapogenoic Acid.**—To 2.1 g. of pure anhydrosarsasapogenoic acid (m. p. 241–243° dec.) suspended in 80 cc. of water was added 20 cc. of 2% sodium hydroxide solution. The mixture was cooled to –5° and an excess of 2% potassium permanganate added dropwise with stirring. The solution was then allowed to remain at room temperature for thirty minutes with an excess of potassium permanganate solution. It was slowly filtered through kieselguhr at room temperature, acidified with dilute sulfuric acid and the precipitated solid was extracted with ether. The ethereal

solution was washed with water and evaporated to a volume of 100 cc. After standing, the fine white crystals were filtered and recrystallized from ether as white prisms, m. p. 285–287° dec. This gave no depression when mixed with an authentic sample of 3( $\beta$ )-hydroxy-16-keto-*bis-nor*-cholanolic acid. A yield of 650 mg. of pure product was obtained.

*Anal.* Calcd. for  $C_{22}H_{34}O_4$ : C, 72.9; H, 9.5. Found: C, 72.6; H, 9.4.

**Alkaline Permanganate Oxidation of the Dibasic Acid Obtained from the Cold Permanganate Oxidation of Anhydrosarsasapogenoic Acid.**—To 250 mg. of pure dibasic acid (m. p. 206–207° dec.) suspended in 20 cc. of water was added 7 cc. of 2% sodium hydroxide solution. To the mixture an excess of 2% potassium permanganate solution was added dropwise, with stirring, at room temperature. It was allowed to stand for thirty minutes. The solution was filtered, acidified with dilute sulfuric acid and extracted with ether. The ethereal solution was washed with water and evaporated, yielding a product which was not purified.

(1) Fieser and Jacobsen, *THIS JOURNAL*, **60**, 2753 (1938).