

2. The yield of oxalic acid increases with an increase in both the temperature employed and an increase in the concentration of the alkali, while that of acetic acid diminishes with a decrease in temperature and an increase in the concentration of the alkali.

3. The production of carbon dioxide increases with the temperature. In neutral permanganate solutions acetone should be oxidized completely to carbon dioxide at about 164°. Acetic acid and carbon dioxide arise from the oxidation of acetaldehyde and methylene, respectively. The presence of oxalic acid in neutral solutions is due to the very slow oxidation of acetic acid and the oxidation of vinyl alcohol.

4. Acetone in aqueous solution must exist in equilibrium with *iso*-acetone. An increase in the concentration of the alkali increases *iso*-acetone formation.

5. Our experiments also confirm the views of Denis and Witzemann that acetol is an intermediate product in the oxidation of acetone.

6. The maximum and minimum effect observed for acetic and oxalic acids, respectively, at higher concentration of alkali in the oxidation of acetol were not observed in our work.

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[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORY, THE UPJOHN COMPANY]

## THE PHYTOSTEROLS OF RAGWEED POLLEN

BY FREDERICK W. HEYL

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In the unsaponifiable fraction of pollen fat from ragweed (*ambrosia artemesifolia*, L.) we have identified traces of a hydrocarbon, along with appreciable quantities of the higher homologs of the paraffin alcohol series. The presence of cetyl ( $C_{16}H_{34}O$ ) and octadecyl alcohol ( $C_{18}H_{38}O$ ) has been established.

The chief interest in this material, however, is found in the sterol alcohols. We have found the phytosterol fraction to be an optically active complex crystalline mixture which resists to a remarkable degree all efforts at separation by the regular methods of fractional crystallization. By persistent recrystallization of the acetates from acetic anhydride, one of these has been satisfactorily characterized. The regenerated phytosterol crystallizes in needles and melts at 147–149°. It agrees with the formula,  $C_{20}H_{34}O \cdot H_2O$ . To this we assign the name "ambrosterol." The corresponding acetate,  $C_{20}H_{33}O \cdot COCH_3$ , forms magnificent plates melting at 112–113°, and has a specific rotation of +27.7°.

Isomeric sterols of this composition have previously been isolated from quebracho,<sup>1</sup> cuprea bark<sup>2</sup> and cinchona bark,<sup>2</sup> Kδ-sam seeds,<sup>3</sup> cascara

<sup>1</sup> Hesse, *Ann.*, **211**, 272 (1882).

<sup>2</sup> Hesse, *ibid.*, **228**, 291 (1885).

<sup>3</sup> Power and Lees, *Year Book Pharmacy*, **1903**, 503.

bark<sup>4</sup> and *Rumex crispus*.<sup>5</sup> All these, however, are levorotatory ( $-29^{\circ}$  to  $-37^{\circ}$ ) and form low-melting acetates. From olive leaves<sup>6</sup> an isomer melting at  $174^{\circ}$  was obtained. Another isomer from colocynth<sup>7</sup> melts at  $158-160^{\circ}$  and has  $[\alpha]_D = +8.1^{\circ}$ . This forms an acetyl derivative that melts at  $167-170^{\circ}$ . From all these ambrosterol differs.

Further we have found in this fraction a quantity of an isomer of the well-known formula,  $C_{27}H_{46}O$ , melting at  $147.5-148^{\circ}$ . Furthermore, there is much evidence of the presence in considerable amounts of a more highly oxygenated substance, perhaps an oxyphytosterol,  $C_{27}H_{46}O_2$ .

### Experimental Part

The unsaponifiable matter of the fat from 800 g. of ragweed pollen was prepared from the crude ether extract. The ether extract was shaken with the usual alkaline solutions. The neutral fat was then saponified with 200 cc. of alcoholic potassium hydroxide by boiling for 5 hours under a reflux condenser. The alcohol was removed, and the residue diluted with 500 cc. of water. The unsaponifiable material was extracted with ether, and dried over anhydrous sodium sulfate. Upon removing the solvent, 51 g. of an orange-colored oil was isolated (6.4%).

This residue was dissolved in 1.5 liters of absolute alcohol. Upon concentration to about 800 cc. there separated a small quantity (0.05 g.) of material melting at  $58-60^{\circ}$ . Recrystallized once from ethyl acetate, the material softened at  $60^{\circ}$  and melted at about  $68^{\circ}$ . It is, therefore, a characteristic mixture of a hydrocarbon and a higher alcohol.

The filtrate was gradually concentrated to a volume of 250 cc. and 2.3 g. of fluffy needles separated. Recrystallized from alcohol these melted at  $148-148.5^{\circ}$  rather sharply.

*Analyses.* C, 81.9, 81.7; H, 11.6, 11.5.

The main filtrate from which the above 2.3 g. had separated was concentrated to about 150 cc. when a second crop (1.1 g.) separated. This was recrystallized from the mother liquors of the first material.

The main filtrate was now concentrated to a sirup and a third crystalline deposit (1.0 g.) was formed. This was recrystallized from the mother liquor of the second crop; m. p.,  $149^{\circ}$ .

*Analysis.* Subs., 0.1393:  $CO_2$ , 0.4225;  $H_2O$ , 0.1467. Calc. for  $C_{20}H_{34}O$ : C, 82.8; H, 11.8. Found: C, 82.7; H, 11.8.

The total yield of phytosterols amounts to 0.55% of the pollen.<sup>8</sup>

This phytosterol appeared to agree well with one having the composition,  $C_{20}H_{34}O$ . It was, nevertheless, a complex mixture, as shown by the following results obtained by systematic fractionation from alcohol. Nine fractions resulted and the top one after 5 recrystallizations remained a mixture.

Fract.	M. p. °C.	Composition		Fract.	M. p. °C.	Composition	
		C	H			C	H
2	147-149.5	81.1	11.3	5	146-149	80.3	11.3
3	146.5-149.5			6	147-149	83.4	12.1
4	149.5-150.5			8	.....	82.3	11.8

<sup>4</sup> Jowett, *Proc. Am. Pharm. Assoc.*, **52**, 288 (1904).

<sup>5</sup> Beal and Okey, *THIS JOURNAL*, **41**, 700 (1919).

<sup>6</sup> Power and Tutin, *J. Chem. Soc.*, **93**, 895 (1908).

<sup>7</sup> Power and Moore, *ibid.*, **97**, 108 (1910).

<sup>8</sup> Koessler, *J. Biol. Chem.*, **35**, 415 (1918), obtained 0.34% by the Windaus method.

*Analyses.* Calc. for  $C_{27}H_{46}O_2$ : C, 80.6; H, 11.4. Calc. for  $C_{27}H_{46}O$ : C, 83.9; H, 11.9. Calc. for  $C_{20}H_{34}O$ : C, 82.8; H, 11.8.

From these data it appears probable that a compound of the nature of an hydroxy-phytosterol,  $C_{27}H_{46}O_2$ , was present in the upper fractions. The fact that Fraction 6 approximates the formula  $C_{27}H_{46}O$  may be taken as proof of the presence of an isomer of this very commonly occurring substance. The subsequent decrease in the carbon content in Fractions 8 and 9 indicates the presence of an isomer of phytosterol having the formula  $C_{20}H_{34}O$ .

The presence of a phytosterol having the composition  $C_{27}H_{46}O$  was further established as follows. A fresh quantity (30 g.) of unsaponifiable material was prepared and from this 2.8 g. of the crude phytosterol fraction was obtained (m. p. 137–142°). It was heated with a volume of absolute alcohol just insufficient to dissolve all of the material, and filtered while hot. The insoluble needles (0.2 g.) melted very sharply at 147.5–148°. It was recrystallized and analyzed.

*Analyses.* Subs., 0.0976; dried at 110° in a vacuum; loss, 0.0045. Calc. for  $C_{27}H_{46}O.H_2O$ :  $H_2O$ , 4.8. Found: 4.6. Subs., 0.0931:  $CO_2$ , 0.2846;  $H_2O$ , 0.0961. Calc. for  $C_{27}H_{46}O$ : C, 83.9; H, 11.9. Found: C, 83.4; H, 11.6.

The filtrate from this compound showed  $[\alpha]_D = +13.7$ , and the same rotation was found for the remaining part of the material which had been previously studied.

They were joined and acetylated. The mixed acetates were fractionally crystallized from acetic anhydride. The top fraction was recrystallized 8 times and the volumes of acetic anhydride used were large, amounting to 100 cc. or more for the first 3 or 4 crystallizations. The first separations, when examined under the microscope were apparently mixtures, but gradually plates with indistinct edges predominated and those finally sharpened, and extremely definite homogenous plates resulted. The first 3 fractions were identical, weighed 0.26, 0.3 and 0.3 g., respectively, and melted at 112–113°.

*Analyses.* Subs., dried at 100° in a vacuum, 0.1217:  $CO_2$ , 0.3569;  $H_2O$ , 0.1186. Calc. for  $C_{22}H_{36}O_2$ : C, 79.5; H, 10.9. Found: C, 79.95; H, 10.9. Subs., (acetate) 0.3 made up to 20 cc. with chloroform, in a 2cm. tube, gave  $\alpha = +0.83^\circ$ , whence  $[\alpha]_D = +27.7^\circ$ .

Subs. (acetate) 0.3, boiled for 1 hr. with 20 cc. of alcoholic potash. Calc. for acetic acid equivalent (mono-acetate): 9.1 cc. of 0.1 N alkali. Found: 7.2 cc.

The regenerated phytosterol was crystallized thrice from 95% alcohol, from which it deposited in homogeneous needles melting at 147–149°.

*Analyses.* Subs., 0.1236, heated at 110° in a vacuum: loss, 0.0065. Calc. for  $C_{20}H_{34}O.H_2O$ :  $H_2O$ , 5.0. Found: 5.2. Subs., 0.1171:  $CO_2$ , 0.3527;  $H_2O$ , 0.1224. Calc. for  $C_{20}H_{34}O$ : C, 82.7; H, 11.7. Found: C, 82.2; H, 11.7.

When a trace of this substance is boiled with 10 drops of acetic anhydride, and the solution cooled and diluted with 10 cc. of chloroform, a fairly persistent deep violet or blue color is obtained upon the addition of a drop of conc. sulfuric acid.

The intermediate fractions of the acetates were obviously mixtures, but in Fractions 7, 8 and 9 an abundance of prisms was obtained. It was thought that these represented a separation and this group was fractionated separately. Analysis proved that a fraction, perfectly homogeneous under the microscope, melting at 105–108°, was simply an impure mixture in which the above described acetate predominated;  $[\alpha]_D = +24.8^\circ$ .

*Analysis.* Subs., 0.0847:  $CO_2$ , 0.249;  $H_2O$ , 0.0818. Found: C, 80.2; H, 10.9.

The regenerated phytosterol melted at 146–147°, and when mixed with the one described above, the melting point was unchanged.

The alcoholic sirupy mother liquors were now freed from alcohol and distilled under

diminished pressure. The following fractions were made: (1) 120–180° at 20 mm.; (2) 180–220° at 15 mm.; (3) 220–290°; (4) 290–320°, (5) above 320°. The first 3 fractions were systematically fractionated at 15 mm., and 2 main fractions collected: (1) 170–190°; (2) 190–196°. The boiling point of cetyl alcohol at this pressure is 189.5°. The latter fraction melted at 49–51° after one recrystallization from alcohol.

*Analysis.* Calc. for  $C_{16}H_{34}O$ : C, 79.3; H, 14.0. Found: C, 79.9; H, 13.45.

This material is apparently impure cetyl alcohol. Repeated recrystallization from alcohol tended to separate an alcohol agreeing well with the composition of octodecyl alcohol.

*Analysis.* Calc. for  $C_{18}H_{38}O$ : C, 80.0; H, 14.1. Found: C, 80.1; H, 13.7.

### Summary

The unsaponifiable fraction of ragweed pollen has been systematically examined, and found to contain the following substances: (1) "am-brosterol," a new phytosterol  $C_{20}H_{34}O$  melting at 147–149° (the corresponding acetate melted at 112–113°), (2) a phytosterol,  $C_{27}H_{46}O$ , (3) cetyl alcohol, (4) octodecyl alcohol, (5) traces of hydrocarbon. Some evidence has been obtained indicating the presence of an hydroxyphytosterol.

The writer is indebted to Mr. Merrill C. Hart and Mr. Clayre Pomeroy for the analytical work.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

## CATALYSIS OF THE FORMATION AND HYDROLYSIS OF ACETAMIDE BY ACETIC ACID<sup>1</sup>

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In 1884 Menschutkin<sup>2</sup> studied the formation of several amides by heating ammonium acetate and other ammonium salts of organic acids in sealed tubes at various temperatures. He determined the amount of amide formed in 1 hour at various temperatures, and called this the "initial velocity" of the reaction. It is evident that such an expression is very inaccurate, since the amounts formed varied from 6.33% at 125° to 82.83% at 212.5°. He also determined the "limit" of the reaction, that is, the amount of amide formed when the reactions for the formation and hydrolysis of the amide are in equilibrium. Ostwald<sup>3</sup> also studied the hydrolysis of acetamide by different acids. His work was chiefly of interest in determining the "strength" of the acids, before the modern theory of ionization was developed. So far as we are aware, no other studies of the velocities of formation and hydrolysis of amides have been made.

<sup>1</sup> A part of the results given in this article was made the basis for a thesis presented to the Graduate Faculty of the University of Illinois by Walter F. Goebel in partial fulfillment of the requirements for the degree of Master of Arts.

<sup>2</sup> Menschutkin, *J. prakt. Chem.*, **29**, 422 (1844).

<sup>3</sup> Ostwald, *ibid.*, **27**, 1 (1883).