The advantages of this modified method are that the alkalies are entirely freed from barium in one operation and are transformed into sulphates, which do not present the difficulties of ignition found with the chlorides.

UNIVERSITY OF ILLINOIS AGRICULTURAL
EXPERIMENT STATION.

DOES CHOLESTEROL OCCUR IN OLIVE OIL?

BY AUGUSTUS H. GILL AND CHARLES G. TUFTS.

Received February 20, 1903.

UPON this question there has been much uncertainty. Beneke[1] stated in 1862 that he believed he had obtained cholesterol from olive oil though the amount was too small to admit of proof. Salkowski[2] in 1887 found the amount obtainable from 50 grams too small for a determination of the melting-point, though the color reactions of phytosterol were given, and he expressed the opinion that olive oil contained neither phytosterol nor cholesterol but that the small amount present in the sample examined by him was due to the presence of olive kernel oil. Gerard[3] reported in 1892 that he had obtained phytosterol from olive oil and had identified it by the melting-point. Bömer[4] declared in 1898 that from two samples of Provence oil he had obtained an alcohol whose melting-point and crystal form left its identity with phytosterol beyond question. In the same year, however, Lewkowitsch[5] stated definitely that "The unsaponifiable matter occurring in olive oil is cholesterol, whereas all other vegetable oils contain phytosterol," and in 1898, Allen[6] also stated that "it (cholesterol) occurs in olive oil" and again that "Phytosterol is found in most vegetable oils with the notable exception of olive and palm." That Allen was not unfamiliar with the work of Bömer seems evident from the fact that he refers to the article in which the latter's work is described. Soltsien in 1901 reported the detection of phytosterol in olives from Bari pressed by himself.[7]

In view of the fact that the matter was of interest not only from

---

[1] *Ann. Chem. Pharm.*, **122**, 249-255.

[2] *Ztschr. anal. Chem.*, **26**, 581.

[3] *Compt. rend.*, **114**, 1544-1546.

[4] *Ztschr. Unter. Nahr. u Genus.* (1898), pp. 81-96.

[5] "Chemical Analysis of Oils, Fats and Waxes," second edition, p. 452.

[6] "Commercial Organic Analysis," third edition, Vol. II, Part 1, pp. 347 and 351.

[7] *Chem. Centrbl.*, **72**, II, 243 (1901).

a purely scientific standpoint but also might be of importance in the detection and analysis of an important oil, it seemed desirable that the question should receive further consideration.

The oil first examined was the ordinary green oil of commerce used as a wool oil. Three portions, each of 1.5 kilos, were treated according to the method described in our article upon maize oil.[1] The unsaponifiable matter so obtained was small in amount and appeared to consist largely of coloring-matter and other substances quite dissimilar from either phytosterol or cholesterol. After several saponifications with alcoholic potash and repeated washing with water, the residue was a highly colored semiliquid grease from which no crystals could be obtained either by precipitation, successive treatment with small portions of alcohol, or slow evaporation from solution in either strong or dilute alcohol. The color reactions of this group of compounds were given in all cases. The residues from the three portions of oil were combined and boiled an hour with an excess of benzoyl chloride; the product was washed with small portions of alcohol and saponified. After two recrystallizations, the partially purified alcohol was boiled with an excess of acetic anhydride; on washing the resulting acetate with alcohol and then saponifying, a crystalline substance was obtained which, though small in amount and still too impure for a determination of the melting-point, consisted of the easily recognizable six-sided plates characteristic both of phytosterol and of sitosterol.

As the evidence afforded by the above examination was inconclusive, it seemed important to obtain an olive oil which should contain the substance sought in greater quantity; it was thought that this would be the case with oils drawn from the most mature fruits. An oil was therefore chosen which had been pressed from fully ripened olives; it was a Californian virgin oil of a light yellow color and strong heavy taste. The specific gravity at 15° C. was 0.917 and the iodine value 83.5; the oil was of known origin and quite free from any adulteration.

One kilo of this oil, extracted as before, yielded at once a light yellow crystalline mass which, after a second saponification and washing, was recrystallized from alcohol. The yield of impure crystals was 0.093 per cent.

To determine whether the finally purified alcohol was a single

---

[1] This Journal, **25**, 254.

compound or had been mixed with other substances, the mother-liquors from the various recrystallizations were themselves recrystallized. All yielded crystals identical in form; the melting-points were a trifle lower than that of the main portion, but this may be due to the fact that as the amounts were smaller they could not be quite as completely purified. The variation was in no case greater than 2°. The crystals obtained from the mother-liquors were used in preparation of the esters as well as the main portion.

The bromide was made by treatment of the solution in carbon tetrachloride with a dilute solution of bromine: the action was slow. Half an hour after the bromine had been added, apparently in slight excess, the solution was completely decolorized and two further additions of bromine were followed by the same result. The melting-point was not determined, as purification was difficult and the compound turned black on standing.

The acetate was prepared by boiling an hour with an excess of acetic anhydride, and formed oblong flat plates melting at 120.3°-120.7°.

The propionate was prepared similarly from propionic anhydride and also formed oblong plate crystals: the melting-point was 102.5°-103.5°.

The benzoate, like the preceding esters, was made from the anhydride and formed oblong plates; it was noticed that in some cases the longer sides were apparently not quite parallel. The melting-point was 145°-145.5°.

The melting-point of the alcohol itself, even when pure, was found to vary somewhat with the conditions under which it was taken. When first prepared and dried at 100°, the melting-point was 134°-134.5°; some days later, after exposure to the air, it was found to be 132°-133°. To examine this depression more closely, the propionate, which had been carefully purified, was saponified; the product rapidly recrystallized five times by chilling the solution in hot alcohol, and the crystals were then dried at 100°. The melting-point, determined immediately, was 135°-135.5°; after twenty-four hours it was found to be 133°-133.5° and after seventy-two hours 132.5°-133°. In this connection it may be of interest to state that although no such rapid fall was noticed in the case of the sitosterol from maize oil, a portion of this latter substance, which originally melted at 138°, examined about eighteen months after preparation, having been protected meanwhile from light, showed a melting-point of 128°-130°.

A sample of phytosterol from olive oil which had been prepared by twice saponifying the propionate melting at 101.5°-103.5° and recrystallizing the product three times, showed, two weeks after preparation and after five days' heating in a dark closet at about 90° C., a melting-point of 99°-103°; the melting-point of the same sample after about six hours' heating was 127°-129°. The phytosterol showed as a result of the longer heating a faint yellow color. The melting-point, in all cases, was determined in a straight capillary tube attached to the stem of a small bulbed thermometer suspended in a 150 cc. flask of concentrated sulphuric acid. The flask was supported about an inch above an iron plate which was heated by a small flame. The temperature was brought fairly rapidly to within 15° to 20° of the melting-point and the rise was then reduced to about 2° a minute. When within 2° or 3° of the melting-point, the rate was made about 0.2° a minute and was there maintained until melting was complete. This observed depression of the melting-points may perhaps explain the varying values recorded in the literature. The crystal form of the alcohol from olive oil closely resembled that of the sitosterol from maize oil. The color reactions of the two compounds were the same. The identification of the compound is the more difficult because observers have disagreed upon the melting-points of these alcohols as well as upon their esters.

The following table shows the melting-points which would seem to be the most trustworthy given by various investigators for phytosterol, sitosterol, cholesterol and the body from olive oil, and their esters.

	Cholesterol.	Phytosterol.	Sitosterol.	Alcohol from olive oil.
Alcohol	146°–147°[1]	132°–136°[4] 136–141.5[5]	137.5°[2] 136.5[3] 138[9]	135°–135.5°[9] 135–136.5[10] 135.5–136.5[11]
Acetate..........	113–114[2]	120[6] 117–126[7] 123.5–135[8] 120–121[9]	127[3] 127.1[9]	120.3–120.7[9]
Benzoate	145–145.5[3]	142–146[8] 144–147[7]	145–145.5[2,3] 142–142.5[9]	145–145.5[9]
Propionate	97–98[2]	104–116[8]	108.5[2] 108.4[9]	102.5–103.5[9]

The melting-points in the above table would seem conclusive evidence that the alcohol from olive oil is not cholesterol. Though the benzoates of the two compounds have the same melting-point, the olive oil compound did not show the double melting-point characteristic of cholesterol benzoate and gave oblong plate crystals, while cholesterol benzoate crystallizes in quadratic tables.[1] Furthermore, the propionate was carefully examined for the play of colors on cooling, stated by Obermüller[12] to be an easily recognizable and characteristic test for cholesterol, and no colors were detected. The difference in the manner of growth and in the crystalline form of the olive oil alcohol and of the cholesterol from wool grease, which was examined simultaneously, is quite unmistakable.

The further identification is rendered difficult by the fact that under the general term "phytosterol" have been included alcohols which it seems probable are not identical. Thus the alcohols from rapeseed oil and from cottonseed oil are both called "phytosterol," though the melting-points of the acetates are in one case 134°-135° and in the other 123.5°-124°.

In the following table are brought together the values for sito-

---

[1] Reinitzer : *Monatsh. Chem.*, **9**, 421 (1888).
[2] Mauthner and Suida : *Ibid.*, **15**, 367 (1894).
[3] Ritter : *Ztschr. physiol. Chem.*, **34**, 461.
[4] V. Lipman : *Ber. d. chem. Ges.*, **20**, 3201 (1887).
[5] Hesse : *Ann. Chem.* (Liebig), **192**, 175.
[6] Hesse : *Ibid.*, **228**, 296 (1885).
[7] Jacobsen : *Ztschr. physiol. Chem.*, **13**, 32 (1889).
[8] Bömer and Winter : *Ztschr. Unter. Nahr. u. Genus.*, **4**, 864 (1901).
[9] Gill and Tufts : This Journal, **25**, 254.
[10] Gerard : *Compt. Rend.*, **114**, 1544 (1892).
[11] Bömer : *Ztschr. Unter. Nahr. u. Genus.*, (1898), p. 81.
[12] *Ztschr. physiol. Chem.*, **15**, 39 (1891).

sterol from maize oil and from wheat, for the alcohol from olive oil, and for the phytosterol from cottonseed oil.

	Sitosterol.	Alcohol from olive oil.	Phytosterol.
Alcohol	136.5°–138°[1,2,3]	135°–136.5°[4,3]	136°–137°[5]
Acetate	127[1,3]	120.3–120.7[3]	123.5–124[5] 120–121[3]
Propionate	108.5[1,3]	102.5–103.5[3]	104–105[5]
Benzoate	142–145.5[1,2,3]	145–145.5[3]	142–143[5]

While the differences here are less marked than in the case of the olive oil alcohol and cholesterol, the melting-points seem, on the whole, to indicate that the compound from olive oil resembles the phytosterol from cottonseed oil more than sitosterol from maize oil or from cereals, and is undoubtedly phytosterol and not cholesterol.

THE DETERMINATION OF BENZENE IN ILLUMINATING GAS.

BY L. M. DENNIS AND J. G. O'NEILL.

Received February 8, 1903.

IN 1891 Hempel and Dennis described a method[6] for the volumetric determination of certain hydrocarbons that are usually present in illuminating gas. Up to that time, all hydrocarbons in this product, with the exception of methane, had been determined by absorption with fuming sulphuric acid and had been classed under the general term "heavy hydrocarbons." It is true that Bunsen[7] gives an analysis of illuminating gas in which the percentages of benzene, ethylene and propylene are stated, but the amounts of these three gases were calculated by means of equations from the results of explosions with air and oxygen, and the calculation was based upon the assumption that the heavy hydrocarbons in the gas consisted only of ethylene, propylene and benzene. It was ascertained by Hempel and Dennis that certain hydrocarbons such as benzene and naphthalene could be removed, in part at least, by means of absolute alcohol, the remainder of the heavy hydrocarbons being then absorbed by fuming sulphuric acid and the methane being finally determined by explosion or combus-

---

[1] Mauthner and Suida: *Monatsh. Chem.*, **15**, 367 (1894).
[2] Ritter: *Ztschr. physiol. Chem.*, **34**, 461.
[3] Gill and Tufts: This Journal, **25**, 254.
[4] Bömer: *Ztschr. Unter. Nahr. u. Genus.* (1898), p. 81.
[5] Bömer and Winter: *Ibid.*, **4**, 864 (1901).
[6] *Ber. d. chem. Ges.*, **24**, 1162.
[7] "Gasometrische Methoden," second edition, (1877), p. 142.