

green, permanent for twenty-four hours. Though these reactions and those of the high-melting alcohols of wool grease are too much alike to be of value in analysis, the following differences were observed. On standing, the chloroform layer of Salkowski's test is blood-red with a concentrated solution of wool grease alcohol, and purple with the alcohol from maize oil. With Lieberman's test, in dilute solution, wool grease alcohol gives a bluish green which becomes reddish yellow on standing, while maize oil gives a clear green changing, on standing, to a yellow free from red.

The above results seem sufficient to prove that the alcohol of maize oil is not cholesterol. As shown by the following comparative table, the compound studied is undoubtedly identical with the compound found in wheat and rye and described by Burian under the name "Sitosterol."<sup>1</sup> It is also probably identical with the "Hydrocarotin" of Reinitzer.<sup>2</sup>

#### MELTING-POINTS OF SITOSTEROL AND ITS ESTERS.

Sitosterol.	From wheat.	From maize oil.	Hydrocarotin.
Sitosterol. ....	137.5°	138°	137.4°
" acetate. ....	124.5°-127° <sup>3</sup>	127.1°	127.6°
" benzoate ...	145.0°-145.5°	142.0°-142.5°	144°
" propionate .	108.5°	108.4°	....

### SITOSTEROL, A POSSIBLE TEST FOR MAIZE OIL.

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WHILE the presence of cottonseed oil in other oils is easily detected, the recognition of adulteration of cottonseed oil itself is less simple. The addition of many oils would be shown by their effect on the usual analytical constants, but with maize oil these values show so little divergence from those of pure cottonseed oil that detection of admixture is difficult. Since, however, maize oil contains sitosterol<sup>4</sup> while cottonseed oil contains phytosterol, it seemed that a test might be based upon this difference.

In the preceding article it was observed that the acetate of sitosterol from maize oil seemed rather less soluble in alcohol than

<sup>1</sup> *Monatsh. Chem.*, **18**, 551-574 (1897).

<sup>2</sup> *Ibid.*, **7**, 597-608.

<sup>3</sup> Softened at 124.5° and melted at 127°.

<sup>4</sup> See the preceding article.

the acetate prepared from olive oil. It was thought that if a relatively small amount of maize oil were to be mixed with an oil known to contain phytosterol, it would be possible to secure by recrystallization of the acetates a portion whose melting-point would approach that of sitosterol acetate. The following trials were therefore made.

A pure cottonseed oil, a cottonseed oil containing 10 per cent. of maize oil, and one containing 20 per cent. of maize oil were extracted, using 50 gram portions, by boiling twenty minutes at a reflux condenser with 100 cc. of 95 per cent. alcohol. The mixtures were then run into separatories and left over night. The clear alcoholic layer was drawn off, boiled fifteen minutes with 75 cc. of half normal alcoholic potash, evaporated to dryness and the residue dissolved in 40-50 cc. of water. The cooled soap solution was shaken out with 75 cc. of ether and 3 cc. of alcohol, and the extract was washed three times with water and evaporated. The yield by this process, which was not expected to give complete extraction, was as follows: Pure cottonseed oil, 0.095 per cent.; 10 per cent. maize oil, 0.12 per cent.; 20 per cent. maize oil, 0.164 per cent.

The crystals obtained were acetylated without further purification by boiling one hour with an excess of acetic anhydride in a beaker covered with a watch-glass containing a little water. The excess of anhydride was evaporated on the water-bath and the acetates recrystallized from alcohol. In recrystallizing, the acetates were dissolved in hot 95 per cent. alcohol and water was then added drop by drop as long as the solution remained clear after shaking; as soon as there were indications of permanent opalescence a few drops of alcohol were added until the solution was perfectly clear. The beaker was then set aside and the crystals subsequently formed were filtered off, dried at 100° C. and the melting-point determined. Proceeding in this way the melting-points shown on the sixth, and subsequent, crystallization were: Pure cottonseed, 120°-121°; 10 per cent. maize, 121.5°-122.5°; 20 per cent. maize, 124°-125°. By dissolving the acetates in hot 95 per cent. alcohol just sufficient for solution and then chilling, there was obtained from the 20 per cent. oil after four crystallizations a portion which showed the melting-point 126°-127°; the melting-point of the acetate from the 10 per cent. oil was unchanged.

The crystals deposited on slow cooling from 95 per cent. alcohol were, in the case of pure cottonseed, transparent flat needles; the 20 per cent. maize oil gave crystals which beside these needles, showed an opaque, white, and more granular form; the crystals from the 10 per cent. oil resembled those from the pure cottonseed oil.

Portions of the crystals obtained from the dilute alcohol, as described above, were allowed to crystallize from a little 95 per cent. alcohol on glass slides and examined microscopically. All three acetates showed massed crystals in branching coral forms and also long narrow plates resembling the crystals of phytosterol and of sitosterol. There seemed to be less difference in the crystal forms as thus examined than appeared to the naked eye in the manner of growth of the crystals when slowly deposited.

It would seem that the above test will detect the addition of 10 per cent. of an oil containing sitosterol to an oil carrying phytosterol. While maize oil is the only commercial oil in which sitosterol has so far been definitely reported, Bömer and Winter<sup>1</sup> have obtained acetates melting at 128° and above from linseed, rapeseed and sesame oils; it seems probable that sitosterol is present in more oils than has hitherto been supposed and consequently that the test can only be regarded as conclusive evidence of the presence of maize oil when the analytical constants show the absence of other adulterants.

As the melting-point varies with the method employed, it will be advisable for the operator to determine the melting-point of phytosterol acetate according to the method he himself uses. The above values were determined in straight narrow tubes attached to the bulb of an Alverginat thermometer and suspended in a test-tube of sulphuric acid. This was in turn suspended in a 100 cc. flask about two-thirds full of acid. The flask was supported about an inch above an iron plate heated by a small Bunsen flame. The melting-point was approached fairly rapidly and when within 2° or 3°, the rate of heating was reduced to about 0.2° per minute. The value obtained by Bömer and Winter for the phytosterol acetate from cottonseed oil is 123°-124°, but it may be stated that the values obtained by Bömer for the melting-point of phytosterol are rather higher than those usually reported.

<sup>1</sup> *Ztschr. Unter. Nahr. u. Genuss.*, (1898), p. 81.