tent by the pyridine-aniline method. These extracts were obtained by percolating the air-dried, powdered bark with the warm solvent, followed by evaporation of the solvent under diminished pressure, below 35°. The residues were finally heated to constant weight in vacuo at 50°, and, with the exception of the acetone extract, were free from solvent odor when weighed. The acetone extract appeared to retain some solvent under these conditions, probably combined with the gossypol as gossypol acetonate.

TABLE III

GOSSYPOL CONTENT OF VARIOUS ORGANIC SOLVENT EXTRACTS OF COTTON-ROOT BARK

Dried bark from upland short staple, Delta and Pineland 11 A variety.

Solvent	Crude Extract percent of air-dried bark	Gossypol* percent of air-dried bark
Petroleum ether (Skellysolve F)	1.81	0.62
Ethyl ether	3.40	0.97
Alcohol, 95%	13.46	0.58
Acetone	4.83	1.30
Chloroform	3.35	0.91

* Pyridine-aniline method.

It will be noted from Table III that while the bark yields much more extractive matter with alcohol than with ether, the latter extract is richer in gossypol and gives a higher percentage yield of gossypol, based on the weight of bark. The best yield, however, is shown by acetone, and preliminary tests on the precipitation of gossypol-acetic acid from the acetone extract show promise of giving better yields of crude gossypol than the described ether extraction method. Whether or not the crude gossypol-acetic acid obtained from the acetone extract lends itself to purification as readily as that from the ether extract has not yet been determined. Chloroform extracts a major portion of the gossypol from cotton-root bark in readily purifiable form, but petroleum ether and alcohol do not appear to be satisfactorily solvents for this purpose. The failure of petroleum ether to extract all of the gossypol is accounted for by the insolubility of gossypol in this solvent. In the case of 95% alcohol, it is thought that gossypol extraction is fairly complete, but that the large amount of alcohol-soluble extractive matter which accompanies it prevents complete separation of the gossypol as the dianilino derivative.

SUMMARY

Cotton-root bark from upland short staple cotton contains up to 1.8 per cent gossypol (air-dried basis). Analyses of other parts (stalk, bark-free root, leaves, squares, immature bolls) of the cotton plant showed little or no gossypol in these structures. The gossypol content of the root bark increases with maturity of the plant, and is highest in roots gathered in the winter after the stalk has dried.

Owing to the absence of oil in the root bark, the gossypol obtained therefrom by ether extraction is more easily purified than that from cottonseed. The experimental recovery of pure gossypol from one sample of root bark was 0.88 per cent.

It is estimated that one acre of cotton will yield 150 kilograms of dry root bark, from which about 1.4 kilograms of pure gossypol can be recovered.

Literature Cited

(1) Adams and Baker, J. Am. Chem. Soc., *61*, 1138 (1939).
(2) Adams and Butterbaugh, ibid., *60*, 2174 (1938).
(3) Adams and Dial, ibid., *61*, 2077 (1939).
(4) Adams, Friedman, Price, Morris, and Kirkpatrick, ibid., *60*, 2160 (1938).
(5) Adams and Geissman, ibid., *60*, 2163 (1938).
(6) Adams and Geissman, ibid., *60*, 2166 (1938).
(7) Adams and Geissman, ibid., *60*, 2184 (1938).
(8) Adams and Geissman, ibid., *61*, 2083 (1939).
(9) Adams, Geissman, and Morris, ibid., *60*, 2967 (1938).
(10) Adams, Geissman, and Morris, ibid., *60*, 2970 (1938).
(11) Adams and Hunt, ibid., *61*, 1132 (1939).
(12) Adams, Hunt, and Baker, ibid., *61*, 1134 (1939).
(13) Adams, Hunt, and Morris, ibid., *60*, 2972 (1938).
(14) Adams and Kirkpatrick, ibid., *60*, 2180 (1938).
(15) Adams and Morris, ibid., *60*, 2188 (1938).
(16) Adams, Morris, Butterbaugh, and Kirkpatrick, ibid., *60*, 2191 (1938).
(17) Adams, Morris, Geissman, Butterbaugh, and Kidkpatrick, ibid., *60*, 2193 (1938).
(18) Adams, Morris, and Kirkpatrick, ibid., *60*, 2170 (1939).
(19) Adams, Price, and Dial, ibid., *60*, 2158 (1938).
(20) Campbell, Morris, and Adams, ibid., *59*, 1723 (1937).
(21) Carruth, ibid., *40*, 647 (1918).
(22) Merck's Index, Fourth Ed., 190 (1930).
(23) Miller and Adams, J. Am. Chem. Soc., *59*, 1736 (1937).
(24) Miller, Butterbaugh, and Adams, ibid., *59*, 1729 (1937).
(25) Morris and Adams, ibid., *59*, 1731 (1937).
(26) Power and Browning, Pharm. Jour., *93*, 420 (1914).
(27) Royce and Kibler, Oil and Soap, *11*, 116 (1934).

A Study of Dienometry*

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A CHEMICAL method for the quantitative determination of conjugation in fats and oils was first suggested by Kaufmann and Baltes (1), who found that the conjugated double-bonds present in fatty acids or fats underwent a Diels-Alder reaction when heated with an excess of maleic anhydride solution. The addition product formed was separated from the reaction mixture, and the unreacted maleic anhydride determined by titration. Shortly afterward Ellis and Jones (2) published a rather similar method, claiming increased accuracy and decreased reaction time. This was followed by Kaufmann's iodometric method (3) which differed from the earlier method only in the manner of determination of the excess maleic anhydride. Under the conditions of Kaufmann's methods it is claimed that one mole of the anhydride will react with one double-bond of a conjugated system; so the extent of reaction may be expressed in terms of iodine equivalents. The *diene number* is thus used to express the parts of maleic anhydride, calculated in equivalent amounts of iodine, taken up by 100 grams of fat. Beta-eleostearic acid, for example, is said to have a *diene number* of 91.3. Later work, however, has revealed that maleic anhydride will react not only with conjugated double-bonds but also with certain oxidation products, notably hydroxy compounds (4, 5, 6, 7, 8, 9). Furthermore, McKinney and Jamieson (10) were unable to secure quantitative addition of maleic anhydride to alpha- and beta-eleostearic acids, but instead obtained for both acids a diene number of 78.4, corresponding to 86.6 per cent of the theoretical value.

It is obviously of some practical importance to know the true diene numbers of pure conjugated fatty acids and glycerides, and in this connection the following preliminary study was made in order to ascertain the diene number of beta-eleostearic acid. Since Pelikan and von Mikusch (4) have already shown that

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the Ellis-Jones three-hour method yields higher results with certain compounds and is less independent of reagent excess than the Kaufmann alkalimetric method, the former method will not be considered in this paper.

Experimental

Beta-eleostearic acid was prepared by the method of Thomas and Thomas (11) with increased repetition of crystallization. The melting point (71.5-72) was not affected by additional fractional crystallization from low boiling petroleum ether to insure the absence of hydroxy acids. Alpha-eleostearic acid (M. P. 49-49.5) was prepared in a similar way.

The ethyl ester of beta-eleostearic acid was prepared by a cold esterification using 5N alcoholic-HCl and a 48-hour reaction period. The ester was isolated by pouring the alcoholic solution into ice water, extracting with petroleum ether, washing free of acid, drying, and removing the solvent under nitrogen *in vacuo*. The resulting products was a light-colored oil.

"Wood oil butter" was prepared from a sample of Florida tung oil used in these experiments by adding a few milligrams of sulfur to 500 ml. of the oil and allowing to stand in the light for three days.

Diene numbers were determined by both the iodometric and the alkalimetric methods of Kaufmann. The iodometric procedure was followed without modification with satisfactory results. In the alkalimetric method, however, a persistent emulsion was always produced when the maleic anhydride addition product of the conjugated acid was precipitated from acetone solution by the addition of water. Added salt was comparatively ineffective in breaking the emulsion within 6 to 15 hours, and filtration through qualitative or quantitative filter paper was usually not sufficient to produce a clear filtrate. Filtration through filter paper pulp resting upon No. 42 Whatman filter paper, however, resulted in a clear filtrate, although occasionally a second filtration was necessary. Using this procedure it was not necessary to allow the solution to stand 6 to 8 hours before filtration since the excess maleic anhydride was found to hydrolyze in less than 15 minutes. Actually, filtrations performed 15 to 60 minutes after the contents of the bombs were transferred to flasks gave comparable and somewhat higher diene numbers than were secured with the 6 to 8 hour standing period. The effect of carbon dioxide on the titration was minimized by using carbon dioxide free water, boiling, and titrating the warm solution.

Results

The idodometric diene numbers of samples of beta-eleostearic acid prepared on three separate occasions are given in Table I.

TABLE I.

Iodometric Diene Numbers of Beta-eleostearic Acid

0.1156 g.	64.7	
0.1271 g.	65.6	
0.1088 g.	65.6	
0.1086 g.	65.8	
0.1314 g.	66.0	
0.1232 g.	66.3	
0.1343 g.	66.5	
0.0871 g.	67.2	
0.1025 g.	67.2	
average	66.1	(72.4% of theory)

The same samples, however, exhibited varying values when determined by the Kaufmann alkalimetric method, depending largely upon the care taken to insure a clear filtrate. An average of the best values obtained by omitting the 6 to 8 hour standing period and titrating only perfectly clear solutions gave a diene number of 74.6 (81.7% of theory) for beta-eleostearic acid.

The ethyl ester of beta-eleostearic acid had an alkalimetric diene number of 66.5 and 65.6 (average 66.1), which is equivalent to a diene number of 72.7 (79.6% of theory) for the free acid.

A few determinations of alpha-eleostearic acid indicated that it behaved similarly to the beta-acid with respect to maleic anhydride addition.

The diene number of tung oil was 65.3 determined alkalimetrically or 67.3 determined iodometrically. These values represent the average of over a dozen separate determinations. Tung butter appeared to have the same diene number as tung oil.

Conclusions

Using the Kaufmann alkalimetric and iodometric methods for the determination of conjugation, theoretical values for alpha- and beta-eleostearic acids could not be obtained although the values found for tung oil agreed with those generally accepted. The alkalimetric method was found to give less consistent results than the iodometric method due to the persistent emulsion formed at one stage in the former method making filtration difficult. However, the use of a pad of filter paper pulp resting upon No. 42 Whatman paper avoided this difficulty, giving clear filtrates of higher diene number. Further work is evidently necessary to determine the empirical values that should be used in the calculation of the amount of conjugated doublebonds present in fatty acids or glycerides.

BIBLIOGRAPHY

1. Kaufmann, H. P. and Baltes, J., Fette u. Seifen, *43*, 93-7 (1936).
2. Ellis, B. A. and Jones, R. A., Analyst, *61*, 812-16 (1936).
3. Kaufmann, H. P., Baltes, J. and Büter, H., Ber., *70B*, 903-11 (1937).
4. Pelikan, K. A. and von Mikusch, J. D., Oil and Soap, *14*, 209-10 (1937).
5. Bruce, R. J. and Denley, P. G., Chem. and Ind., *56*, 937 (1937).
6. Bickford, W. G., Dollear, F. G. and Markley, K. S., J. Am. Chem. Soc., *59*, 2744 (1937).
7. Sabetay, S. and Naves, Y. R., Bull. soc. chem. (5), *4*, 2105 (1937).
8. Bickford, W. G., Dollear, F. G. and Markley, K. S., Oil and Soap, *15*, 256-59 (1938).
9. Tyutyunnikov, B. and Ursulyak, S., Masloboino Zhirovoe Delo, *14*, No. 5, 12-15 (1938).
10. McKinney, R. S. and Jamieson, G. S., Oil and Soap, *15*, 30-32 (1938).
11. Thomas, A. W. and Thomas, J. C., J. Am. Chem. Soc., *56*, 898 (1934).