[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN]

The Chemical Examination of the Seed of Abies Balsamea (L) Miller[1,2]

BY S. R. BENSON AND H. N. CALDERWOOD

Introduction

Although the balsam fir, *Abies balsamea* (L) Miller, grows over a large area of North America[3] and is cut extensively for pulpwood the only chemical examination of its seeds is the work of E. von Tubeuf[4] giving one photomicrograph of a cross-section of the seed showing the starch, protein and fat content as determined by histological methods. This paper is the first report in the literature upon the proximate composition of the seed and the characteristics of its fatty oil.

The seed contains noticeable quantities of oleoresin, situated between the placenta scale tissue of the wings or husk and the endosperm, and raised a problem not encountered in securing the common fatty oils from seeds.

Experimental

Collection of Material.—A total weight of 160 kg. of cones was collected which yielded 35 kg. of pure seed.

Composition of Seed.—The proximate composition of the seed is shown in Table I.

TABLE I

COMPOSITION OF SEEDS OF ABIES BALSAMEA

	As received	Moisture-free basis
Moisture, %	8.61	...
Crude fiber, %	32.14	35.13
Crude protein, %	8.62	9.42
Total ash, %	1.72	1.88
(a) Soluble ash, %	0.62	0.67
(b) Insoluble ash, %	1.16	1.26
Total water–ethanol extract, %	21.9	23.9
(a) Non volatile fraction, %	17.5	19.1
1. Water soluble, %	4.06	4.43
2. Water insoluble, %	13.44	14.69
(b) Volatile fraction, %	4.4	4.8
Petroleum ether extract, %	10.69	11.68

The best menstrum for obtaining the oleoresin free from fatty oil was found to be 75% alcohol. The air-dried unground seeds were extracted by percolation at room temperature, approximately 25°. The extract yielded a volatile oil with the following characteristics: b. p. 155–180° (740 mm.); sp. gr.$^{25}_{25}$, 0.8706; n^{25}D, 1.4721; $[\alpha]^{25}$D, −25.32. In addition to the volatile oil, glucosides, tannins and resins were recovered, which will be investigated and reported in later publications.

The husk and endosperm, separated readily in the dry oleoresin-free seed, represented 16 and 60%, respectively, of the total seed. The endosperm after grinding was extracted with petroleum–ether, b. p. 60–65°, and yielded after complete removal of the solvent a clear golden yellow bland fatty oil. The oil yield based upon the endosperm material is 19.8%.

Fatty Oil.—The fatty oil was examined by recognized procedures[5] and these results are summarized in Table II.

TABLE II

CONSTANTS OF FATTY OIL FROM BALSAM FIR SEED

Sp. gr. 25°/25°	0.9279
Ref. index at 25°	1.4783
Solidifying point	−37.0°C.
Titer test	−17.5°C.
Acid value	0.88
Iodine value (Hanus)	141.0
Saponification value	185.6
Acetyl value	15.4
Per cent. unsaponifiable	4.8
Reichert–Meissl number	0.53
Polenske number	.21
Soluble acids	trace
Insoluble acids	
Per cent.	83.2
Thiocyanogen value	93.3
Saturated acids (lead salt–ether method)	
Per cent.	1.6
Melting point, °C.	56
Iodine value	14.0
Mean molecular weight	282.0
Unsaturated acids (lead salt–ether method)	
Per cent.	80.8
Iodine value	155.5
Mean molecular weight	279.0

The unsaturated acids were examined using the bromination method of Eibner and Müggenthäler[6] and the thiocyanogen method of Kaufman.[7] These results are reported in Table III.

A further study of the individual unsaturated acids was made by converting them into hydroxy acids by the method of Hazura.[8] The oxidation products upon separation yielded the hydroxy acids reported in Table IV.

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(1) A portion of a dissertation submitted to the Graduate School of the University of Wisconsin by S. R. Benson in partial fulfilment of the requirements for the Ph.D. degree, June, 1935.

(2) Presented in part before a joint meeting of the Wisconsin Sections of the American Chemical Society and the Wisconsin Academy of Sciences, Arts and Letters, Beloit, Wisconsin, April 27, 1935.

(3) (a) Raphael Zon, "Balsam Fir," U. S. Dept. Agr. Bull., **55** (1914); (b) Geo. B. Sudworth, "Check List of Forest Trees of North America, Their Names and Ranges," U. S. Dept. of Agr. Miscl. Circular, **92**, 29 (1927).

(4) E. von Tubeuf, *Naturwiss Z. Forst-Landw.*, **15**, 231 (1917).

(5) A. O. A. C. Methods of Analysis, 3d ed., Washington, D. C., 1930, pp. 314–330.

(6) Eibner and Müggenthäler, *Farben. Ztg.*, **18**, 131 (1912).

(7) Kaufman, *Z.-Nahr. Genussm.*, **51**, 15 (1927).

(8) Hazura, *Monatsh.*, **8**, 147, 156, 260 (1887); **9**, 180, 190, 469, 478, 944, 947 (1888); **10**, 190 (1889).

TABLE III

EXAMINATION OF THE UNSATURATED ACIDS

Acid	Bromine method	Thiocyanogen method
Linolenic acid, %	3.1	5.0
Hexabromide, m. p., °C.	179	
Bromine in hexabromide, %	63.0	
Linoleic acid, %	54.6	59.7
Insoluble tetrabromide, m. p., °C.	107–109	
Bromine in tetrabromide, %	54.0	
Oleic acid, %	40.0	33.3

TABLE IV

HYDROXY DERIVATIVES ISOLATED BY PERMANGANATE OXIDATION

Acid	M. p., °C.	Neutral equivalent Obsd.	Neutral equivalent Calcd.
Dihydroxystearic	130	315	316
Tetrahydroxystearic	152	349	348
Tetrahydroxystearic	173	348	348
Hexahydroxystearic	174	384	380
Hexahydroxystearic	200–203	...	...

Discussion

Although the fatty oils from seed of the botanical family *Pinaceae* are repeatedly reported in the literature to have a terebinthic odor and taste, only a few genera, including *Abies*, have oleoresin vesicles in the seed.[9] In this paper a method is described for separating the oleoresin and fatty oil in *Abies balsamea* seed. The fatty oil was found to be glycerides of stearic, linolenic, oleic and linoleic acids. The mean molecular weight and melting point of the saturated acids demonstrated them to be essentially stearic.

The bromide and thiocyanometric reactions have enabled a quantitative study of the unsaturated acids of the fatty oil. The values, shown in Table III, obtained by the two methods are not identical but are however of the same order of magnitude. The thiocyanometric method is largely mathematical and serves but to give the relative percentages of acids of the oleic, linoleic and linolenic type. The bromide studies in addition to giving the relative amounts of these different types enabled the identification of the linolenic acid in the oil but did not permit a clear cut separation of the linoleic and oleic acids and therefore the hydroxy derivatives of these acids were prepared.

The hexahydroxy- and hexabromostearic acids obtained proved the linoleic acid to be 9,12,15-octadecatrienoic acid.

Two tetrahydroxystearic acids were obtained with melting points identical with those obtained by Nicolet and Cox[10] for the tetrahydroxy acids prepared from a single tetrabromostearic acid, the structure of which has been determined by W. C. Smit.[11] If it is assumed that the oxidation of linoleic acid with permanganate proceeds in a manner similar to that of oleic acid wherein a single olefinic acid yields but one hydroxy derivative, the fatty oil of the balsam fir seed contains two stereoisomeric linoleic acids. Further work upon the structure of these was in progress when the paper by Green and Hilditch[12] appeared wherein they state all seed fats contain but a single linoleic acid. The results of these studies will appear later.

The single dihydroxystearic acid obtained showed, by its melting point and degradation products, the oleic acid present in the oil to be the 9-octadecenoic acid.

It is a pleasure to express our gratitude to Messrs. D. C. Van Ostrand and Bruce G. Buell of the Patten Timber Company, Amasa, Michigan, for their generosity and assistance in obtaining the material used in this investigation.

Summary

1. This paper is the first chemical examination of the seed of *Abies balsamea* (L) Miller reported in the literature.

2. The seed of *Abies balsamea* contains on the moisture free basis 19.8% oleoresin and 11.7% of fatty oils.

3. These substances as far as can be determined by chemical methods are located in separate parts of the seed; the oleoresin between the parchment-like tissue of the wings and the testa, the fatty oil inside the testa and distributed throughout the endosperm.

4. The common physical and chemical constants of the fatty oil have been determined.

5. The fatty oil has been shown to be essentially glycerides of stearic, oleic, linoleic and linolenic acids.

6. The relative amounts of acids of the saturated, oleic, linoleic and linolenic types have been determined.

7. The linoleic acid present was shown to be 9,12,15-octadecatrienoic acid. The oil appears to contain two isomeric linoleic acids which

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[9] C. von Tubeuf, *Naturwiss. Z. Forst-Landw.*, **14**, 356 (1916), and **15**, 18 (1917).

[10] Nicolet and Cox, THIS JOURNAL, **44**, 144–52 (1922).

[11] W. C. Smit, *Rec. trav. chim.*, **49**, 539–51, 675–85 (1930).

[12] Green and Hilditch, *Biochem. J.*, **29**, 1552–63 (1935).

yielded tetrahydroxystearic acids with melting points of 152 and 173°. The oleic acid present has been proven to be 9-octadecanoic acid.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

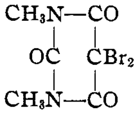
Researches on Pyrimidines. CLI. The Constitution of Dibarbituric Acid[1]

BY ROLLIN D. HOTCHKISS AND TREAT B. JOHNSON

When A. von Baeyer reported in 1864 the preparation of barbituric acid, he described, among its other properties, its conversion by heat into an insoluble new acid, bibarbituric acid.[2] He also reported the isolation of several salts and two bromine derivatives of this new pyrimidine. In the seventy years which have elapsed since that time, barbituric acid and its derivatives have been studied extensively. Dibarbituric acid, however, has received only passing mention by investigators who were dealing with other substances.[3,4] The investigation reported in this paper is concerned with the determination of the structure of this difficultly soluble pyrimidine compound.

Von Baeyer postulated that dibarbituric acid can be represented by the formula $C_8H_6N_4O_5$, corresponding to the union of two molecules of barbituric acid with loss of one molecule of water. This empirical formula is supported by the results now obtained. The literature does not record any attempt to give a structural expression, except that of Conrad and Guthzeit,[3a] who proposed the formulation III.

On treatment with dimethyl sulfate and potassium hydroxide, dibarbituric acid yields a trimethyl and a tetramethyl derivative. When the latter is warmed with bromine water it undergoes bromination and cleavage into two moles of 1,3-dimethyl-5,5-dibromobarbituric acid (I). This fact indicates that the dibarbituric acid molecule contains two intact barbituric acid nuclei united through a linkage not involving the nitrogen atoms. A di-enol anhydride, as represented by formula II would not have the stability toward hydrolysis that dibarbituric acid does, nor would it give *enol* reactions and form salts when all four of the cyclic nitrogen positions have been methylated. The barbituric acid nuclei must, therefore, be linked through carbon atoms, and, since the linkage is easily cleaved by bromine water under the conditions mentioned above, it is probably a double bond. It seems possible, therefore, to exclude all other formulas for dibarbituric acid except III and IV, and their respective tautomeric modifications.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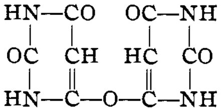
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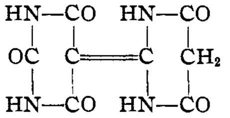
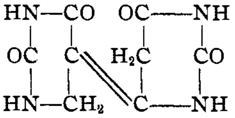

III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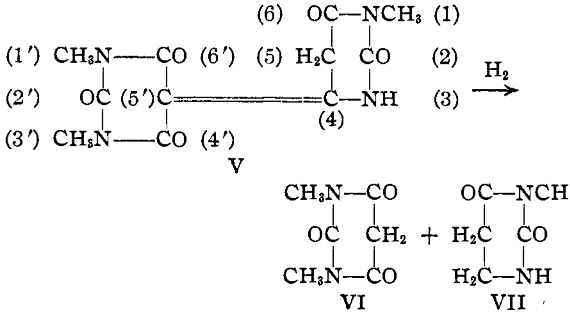
Definite experimental evidence rendering it possible to decide between formulas III and IV was obtained through the hydrogenolysis of N-trimethyldibarbituric acid. Repeated attempts to reduce dibarbituric acid and its N-methyl homologs by a variety of methods failed to result in the preparation of dihydro derivatives. At high temperatures and pressures, however, catalytic reduction of the N-trimethyl derivatives in dry dioxane solution brought about a smooth hydrogenolysis. On the basis of the results obtained from this re-



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[1] From a thesis presented by Rollin D. Hotchkiss to the Graduate Faculty of Yale University in June, 1935, as partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] A. von Baeyer, *Ann.*, **130**, 145 (1864). Throughout this paper the name dibarbituric acid will be used in conformity with modern nomenclature.

[3] (a) M. Conrad and M. Guthzeit, *Ber.*, **15**, 2846 (1882); (b) C. Matignon, *Ann. chim. phys.*, [6] **28**, 292 (1893); (c) H. Biltz and H. Wittek, *Ber.*, **54**, 1035 (1921); (d) H. Biltz and T. Kohler, *ibid.*, **56**, 2482 (1923).

[4] It seems possible that insoluble products observed by Wood and Anderson, *J. Chem. Soc.*, **95**, 979 (1909), and Grimaux, *Bull. soc. chim.*, **31**, 146 (1879), might be impure dibarbituric acid, although these authors did not recognize them as such.