

## SUMMARY.

1. Three new dyes, containing the diaryl sulphide linkage, together with residues present in serenium and pyridium, have been prepared.
2. These substances have been tested for antiseptic activity and have been found to be inactive.

## BIBLIOGRAPHY.

- (1) Hilbert and Johnson, *J. A. C. S.*, 51, 1526 (1929).
- (2) Moness, Braker and Christiansen, *JOUR. A. PH. A.*, 21, 558 (1932).
- (3) U. S. Department of Agriculture, *Circular No. 198*; "United States Food and Drug Administration Methods of Testing Antiseptics and Disinfectants."
- (4) Merz and Weith, *Ber.*, 4, 384 (1871).
- (5) Tchitchibabin and Seide, *J. Russ. Phys. Chem. Soc.*, 46, 1216 (1914).

PHYTOCHEMICAL NOTES.\*<sup>1</sup>

## No. 108. A PHYTOCHEMICAL STUDY OF THE SEED OF THE DIGGER PINE.

BY JOSEPH SEMB.

In the early part of November 1931, forty pounds of Digger pine seed were received from Mr. J. W. Preston, Chico, Calif. One hundred of these seeds were cracked and separated into seed coats and endosperms. The former weighed 53.20 Gm. (= 76.9 per cent), the latter 15.95 Gm. (= 23.1 per cent). Inasmuch as the 100 seeds weighed 69.15 Gm., the average weight of the individual seed is a trifle less than 0.7 Gm. That of the endosperm is about 0.16 Gm.

Samples of ground seed, of seed coats and of endosperm were extracted with selective solvents. The data are recorded below:

## PERCENTAGE EXTRACTED.

Solvent.	From Seed.		From Seed Coat.		From Endosperm.	
	Per Cent.		A (2). Per Cent.	B (3). Per Cent.	C (4). Per Cent.	D (5). Per Cent.
Pet. ether	11.47 (1)		0.18	0.13	53.00	12.20
Ethyl ether	0.27		0.30	0.23	1.00	0.23
Chloroform	0.11		0.00	0.00	0.74	0.17
Alcohol	0.90		0.76	0.59	2.90	0.66
Water	1.98		2.00	1.44	8.20	1.89
1% HCl	15.00		18.20	14.00	27.18	6.26
1% NaOH	2.60		5.00	3.70	..	..

Of the 40 pounds of seed, 37.5 pounds or 17 Kg. were ground in the horizontal disk mill. The coarsely comminuted material was extracted in the cold with petroleum ether for 24 hours. After the percolate had been drawn off, the dregs were reground in a ball mill. This reground residue was subsequently extracted twice with petroleum ether in the cold. A test sample of the last extraction indicated that the extraction was almost complete. Most of the petroleum ether was recovered. The last traces were removed by heating on a water-bath under somewhat reduced pressure for several hours. During part of this time a current of CO<sub>2</sub> was passed through the liquid to assist in the complete removal of the volatile solvent. The petroleum ether extract thus obtained weighed 1950 Gm. = 11.47% of the seed or 49.66% of the endosperm.

\* Scientific Section, A. PH. A., Miami meeting, 1931.

<sup>1</sup> From the laboratory of Edward Kremers.

After having been exhausted with petroleum ether, the ground seeds were percolated with alcohol. The last application of menstruum extracted about 0.15 per cent of the total seed. The recovery of the alcohol yielded a dark brown extract. 100 Gm. of dregs, in four separate experiments were extracted with the following hot solvents yielding the amounts of extract recorded:

1	Chloroform	0.04 Per cent
2	Ethyl alc.	1.13 Per cent
3	Methyl alc.	1.06 Per cent
4	Acetone	0.28 Per cent

As a result of the extractions described the following products were obtained:

- (1) A petroleum ether extract, consisting principally of fatty oil.
- (2) A concentrate of the cold alcoholic percolate.

(1) *The Petroleum Ether Extract (Fatty Oil)*.—This product was almost colorless and odorless and had a bland taste.  $d_{18}^{\circ} = 0.9177$  and  $d_{24}^{\circ} = 0.9167$ ;  $n_{D20}^{\circ} = 1.4713$  (Abbé);  $[\alpha]_D = -0.30^{\circ}$ . It solidified at about  $-15^{\circ}$ .

The oil was perfectly neutral. Three saponification determinations gave 189.9, 190.4 and 189.0, respectively, or an average of 189.8. Its iodine value (Hanus) was found to be 120; its acetyl value 4.9 and 5.0; its thiocyanogen value 83.1, 83.5, 82.3, or an average of 83.0 (6). From these three values, *viz.*, saponification, iodine and thiocyanogen, the following percentages were calculated: 4.3% of saturated fatty acids, 50.5% of oleic acid, and 45.2% of linoleic acid. When one cc. was exposed to the air on a watch glass, an increase in weight of 0.7% was observed after two weeks.

Ninety-seven grams of this oil were oxidized in acetone solution by means of potassium permanganate (7). Four separate fractions were obtained. The fatty acid obtained from the first fraction had a b. p. of  $202^{\circ}$ ;  $n_{D21}^{\circ}$  (Abbé) 1.4146; and a mean molecular weight of 115.1 (calculated from acid value), thus indicating hexoic acid. The second fraction yielded an acid with a b. p. of  $246.2^{\circ}$  and a molecular weight of 148.7 (from acid value). This indicates nonoic acid probably contaminated with hexoic acid. The third fraction yielded an acid which was insoluble in petroleum ether but somewhat soluble in hot water. It had a m. p. of  $106^{\circ}$  and a molecular weight of 188.2 (from acid value assuming a dicarboxylic acid). This indicated azelaic acid. The fourth fraction consisted of 16 and 18 carbon atom fatty acids.

These data indicate that the first double bond in every case is at the 9–10 position, also that this or a second double bond is in the 6–7 or 9–10 position from the end. Had malonic acid been isolated, these data would indicate that only two unsaturated fatty acids are present, that is, the 9–10 oleic and the 9–10, 12–13 linoleic acid. Undoubtedly malonic acid was formed but was lost in the process of purification and isolation.

1625 cc. of the fatty oil were saponified by refluxing with alcoholic KOH. The alcohol was removed by vacuum distillation. About 14 liters of water were added to the potassium soap. The non-saponifiable material was extracted with ethyl ether. Troublesome emulsions were encountered, but the addition of small amounts of methyl alcohol was very effective in breaking these emulsions. Most of the ethyl ether was recovered from the unsaponifiable and the latter was turned over to Dr. Bonstedt.

Dilute sulphuric acid was now added to the soap solution, and immediately a fatty acid ethereal layer (about 3 l.) separated out. (This shows that the soap held a considerable quantity of ethyl ether in solution.) The aqueous layer containing the glycerin was drawn off and shaken twice with ether. The three ethereal portions were united and washed several times with water. The ether and occluded water were removed by heating under vacuum for 2½ hours. The total weight of fatty acid was 1390 Gm. Iodine value 124.2; thiocyanogen value 85.0; acid value 198.1; molecular weight 283.1 (computed from the acid value). Using these three values, the following percentages were calculated: saturated fatty acids 5.5%; oleic acid 51.0%; linoleic acid 43.5%.

1223 grams of this fatty acid were dissolved in 7200 cc. of alcohol. This solution was then heated on the water-bath and to it were added 7200 cc. of alcoholic lead acetate, containing 900 Gm. of the salt (8). The mixture was exposed over night to a temperature of about 6°. The next morning the supernatant liquor was drawn off and the mushy, sticky lead soap adhering to the side of the flask was redissolved in 7200 cc. of hot alcohol which contained a little acetic acid. Again the solution was set outside over night to permit the solid lead soap to settle out, and again the supernatant liquid was drawn off and the solid lead soap redissolved in hot alcohol, the alcoholic solution allowed to cool and filtered. All the alcoholic solutions were mixed. The mixed solutions are supposed to contain the lead soap of the liquid fatty acids. The insoluble lead soap obtained was covered with 400 cc. of ethyl ether. After several days the ethereal solution was drawn off. As will be seen later, the fatty acid recovered from the ethereal solution was added to the free fatty acids from the ether-insoluble lead soap.

*The Solid Fatty Acids.*—The insoluble lead soap was decomposed with dilute HNO<sub>3</sub> and the freed fatty acids extracted with ethyl ether. The ether was removed leaving a solid residue weighing 140 Gm. with a melting point of 31°. The attempt to separate the solid fatty acids by fractional crystallization was without success. Likewise fractional precipitation as magnesium salts was unsatisfactory. The odds and ends were gathered and found to weigh about 100 Gm. To this was added the fatty acid obtained from the ethereal solution that had stood over the alcoholic insoluble lead soap. The methyl esters of this mixture of supposedly saturated acids were made and distilled under 5 mm. pressure.

No.	B. P., ° C.	Wt.	M. P., ° C.	Iodine No.	Saponification Value.
1	...-160	9.8	17	....	....
2	166-170	16.3	18.5	53.3	203.9
3	170-174	26.9	15.5	....	....
4	174-176	25.7	12.0	....	....
5	172 (4½ mm.)	20.68	9.0	84.9	201.7
6	176-182	45.8	12.0		191.1
7	182	15.7	15.0	77.8	
8	Residue	<u>12.1</u>			
		173.0			

The high iodine values may be attributed to imperfect separations. It is well known that none of the methods are any too satisfactory. The methyl ester fraction No. 3 was saponified and the freed fatty acid treated with lead acetate as stated above except that this solution was allowed to cool to only 18°, not to 6°. The insoluble lead soap thus obtained was firm and not mushy as in the original separation. The free fatty acid from this insoluble lead soap had a m. p. of 56°, an iodine value of 5.8, and a mean molecular weight of 261.7 (from the acid value), indicating a predominance of palmitic acid. The freed fatty acid from the alcoholic supernatant liquid gave iodine values of 109.8, 110.8 and 112.8 or an average of

111.2. This indicates the ratio of oleic to linoleic acid to be about the same as that in the original oil (9).

Methyl esters Nos. 1 and 2 were mixed and bromine added at  $-10^{\circ}$  to the petroleum ether solution. The unbrominated saturated fatty esters were separated by reduced pressure distillation. A fatty acid with a m. p. of  $63^{\circ}$  and whose corresponding methyl esters melted at  $28^{\circ}$  was obtained. This indicates that palmitic acid is present. The large amounts of acids obtained at various times from the various fractions with a melting point around  $56^{\circ}$  and a mean molecular weight of about 262 indicates that the greater amount of the saturated fatty acid is palmitic acid.

From fractions Nos. 6 and 7 a fatty acid was isolated, this time by means of the lead-acetate-alcohol method, with a m. p. of  $65.5^{\circ}$ . The methyl ester of this had a m. p. of  $39^{\circ}$ . The mean molecular weight was  $290^{\circ}$  (from acid value). The presence of stearic acid was verified by means of a method suggested by E. Twitchell (10). To 20 parts of the above acid, weighed on an analytical balance, 80 parts of Eastman's stearic acid, also weighed on an analytical balance, was added. The mixture was dissolved in ether and the ether evaporated. The addition of these 20 parts of above acid to 80 parts of Eastman's stearic acid depressed the m. p. of the Eastman stearic acid less than one degree. Therefore, the larger part is stearic acid.

The high molecular weight of the stearic acid fraction discussed above indicates that a saturated fatty acid higher up in the series than stearic is present. From the methyl ester fraction No. 8 a solid fatty acid fraction melting higher than that recorded in the literature for stearic acid was obtained. The quantity was too small to be identified.

*The Liquid Fatty Acids.*—The alcohol-soluble lead soap was decomposed with  $H_2S$ . The freed acids were brominated in acetic acid (11), and from the reaction mixture 208 Gm. of tetrabromstearic acid were obtained. The melting point of this pure white substance, upon recrystallization from petroleum ether, was  $114.0^{\circ}$  and the molecular weight, computed from the acid value, was found to be 608.0. The bromine content, determined by the Stepanow method, was found to be 53.27 per cent. The reported melting point of tetrabromstearic acid is  $114.0^{\circ}$ , the computed molecular weight 600, and the bromine content 53.3 per cent.

The filtrate from the tetrabromstearic acid, which is supposed to contain only the dibromstearic acid was debrominated with zinc. Five grams of the recovered fatty acid were oxidized with dilute  $KMnO_4$ . About one Gm. of a petroleum ether-soluble fatty substance with a m. p. of  $37.0^{\circ}$  was obtained. The petroleum ether-insoluble residue was dissolved in alcohol. Upon cooling, about one Gm. of a white substance with a m. p. of  $131.5^{\circ}$  was obtained. Hilditch reports 9,10 dihydroxy stearic acid to melt at  $132.0^{\circ}$ .

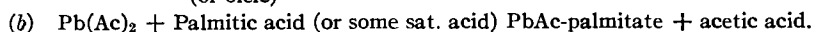
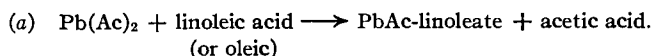
The rest of the liquid acid was steam distilled in order to see if there were any volatile fatty acids present, but the results were negative. This would rule out the presence of any appreciable amount of 6-, 8- and 10-carbon atom acids and possibly a 12-carbon atom acid. According to Hilditch's (*Fats and Waxes*, 74 (1927)) these lower saturated acids might be expected with the unsaturated acids.

Next the methyl esters were made of this liquid acid portion and the esters fractionated under 7 to 8 mm. pressure.

No.	B. P., ° C.	Iodine Value.	Mol. Wt. from Acid Values.		Saponification Value.	
1	-186 (8 mm.)					
2	186-188 (7 mm.)	110.7	290.0		193.4	
		110.3	291.3	290.7	192.1	192.8
3	186-188 (7 mm.)					
4	188-190 (7 mm.)					
5	190	120.5	290.7		193.0	
			291.8	291.3	192.3	192.6
6	190 + residue					

The very small change in boiling point would seem to indicate a very constant boiling constituent. However, the very high iodine value in these esters is noteworthy. The iodine value of oleic acid is 90.0 and that of methyl oleate about 86.0. This would indicate that a great deal of the linoleic acid had not been removed as tetra bromide. This fact was established by isolating tetrabromstearic acid when the freed acid was brominated in petroleum ether solution.

- (1) This percentage is obtained from fat extracted from the main experiment.
- (2) Computed with reference to the seed coat.
- (3) Computed with referenced to the entire seed.
- (4) Computed with reference to the endosperm.
- (5) Computed with reference to the entire seed.
- (6) The method of Zeleny and Baily, *J. I. E. C.*, 24, 109 (1932), was used.
- (7) Hilditch and Vidyarthi, *Royal Society, A.* 122, 552 (1929).
- (8) Hilditch, *Fat and Waxes*, 74 (1927); Twitchell, E., *J. I. E. C.*, 13, 806 (1921).
- (9) Consideration of this fact, that at 6° a great deal of oleic and linoleic acids, in a ratio approximating that in the original, comes down while at 18° very little separates should be worth recalling when this experiment is repeated. If an alcoholic solution of lead acetate is added to an alcoholic solution of fatty acids the following situation arises:



In this particular case reaction "a" predominates and the opportunity of PbAc-palmitate to react with another palmitic acid molecule to yield  $\text{Pb}(\text{palmitate})_2$  is remote compared with its opportunity to react with a molecule of linoleic acid to yield Pb palmitate-linoleate, which undoubtedly is more soluble than  $\text{Pb}(\text{palmitate})_2$  but less soluble than  $\text{Pb}(\text{linoleate})_2$ . The precipitate at 6° was probably a half-saturated and half-unsaturated lead soap such as Pb palmitate-linoleate. At a higher temperature only the saturated lead soaps settle out.

(10) *J. I. E. C.*, 6, 564 (1914).

(11) Rosenthaler-Ghosh, "The Chemical Investigation of Plants," 83 (1930).

## THE LEAF OILS OF WASHINGTON CONIFERS: VII. JUNIPERUS OCCIDENTALIS.\*

BY E. V. LYNN AND LOUIS FISCHER.<sup>1</sup>

*Juniperus occidentalis* Hook. is a tree 20 to 40 feet or more in height. It grows on mountain slopes and high prairies of western Idaho, eastern Washington and Oregon and in the Cascade and Sierra Nevada mountains. Seldom is it found at elevations less than 6000 feet.

The leaves and branches for this work were collected in 1930 near Bend, Oregon. From 600 pounds of fresh material was obtained 986 Gm. of oil by steam dis-

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<sup>1</sup> Seattle, Washington, June 7, 1934.