

served in 16 minutes; in the last case in 120 minutes; in 60 minutes, however, the majority of *Daphnia* lost their ability of body locomotion. An interesting biometric curve was obtained from these data.

CONCLUSIONS.

1. Transparent animals, and especially *Daphnia magna* are well suited for the qualitative and quantitative examination of general physiological effects upon the animal organism and its individual organs.

2. The general effect of strychnine upon *Daphnia* is that of the well-known tonic or stimulating action of small, therapeutic doses; the convulsive action of larger, toxic doses; the paralyzing action of fatal doses, one effect overlapping the other, depending upon particular organs or the difference in vitality of different organisms. Most striking were the effects upon organs of the muscular system; the liver, in agreement with observations recorded for more complex animals, also was visibly effected, through shrinking (3).

3. In a 0.712% solution of strychnine sulphate the heart beat was more effected than the rate of respiration.

4. The time when body immobility (the inability to rise from the bottom of flat test-tubes) is observed in the majority of animals may be used more conveniently—and much more speedily—than the time of death—as a judgment of degree of toxicity.

5. Immobility as well as death are definitely dependent upon concentration of poison in solution and time of action; results are relatively much quicker observed in concentrated than in dilute solutions of strychnine sulphate.

REFERENCES.

- (1) "Transparent Life," by Arno Viehoveer, *American Journal of Pharmacy*, 103 (1931), 252-278.
- (2) "The Heart," by Arno Viehoveer, *Ibid.*, 100 (1928), 718-745.
- (3) "The Strychnine Group," by E. Poulsson; "Handbuch der experimentellen Pharmakologie," pages 322, 331, 392 (1920).

THE OIL FROM THE FRUIT OF MELIA AZEDARACH LINNÉ.*

BY LOYD E. HARRIS AND RALPH M. WILSON.

The oil¹ from the fruit is bitter. It is known as neem oil, Veepa oil, and Veppam fat. It has been used as an anthelmintic² and may be useful as a local application for treatment of rheumatism.

The constants³ of the oil have been reported as follows: Specific gravity, 0.914, (15°); saponification value, 196.9; iodine value, 69.6; Reichert-Meissel value, 1.1; a butyro-refractometer reading of 52°.

EXPERIMENTAL.

A preliminary extraction with a series of selective solvents was made to determine something of the general composition of the fruit. The results are tabulated below: 80 Gm. of the ground air-dried fruit were used for each extraction.

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¹ U. S. Disp., 21st Edition, page 1215.

² Amer. Disp. (King), 8th Edition (1872), page 521.

³ U. S. Disp., 21st Edition, page 1215.

TABLE I.

Solvent.	Sample A. Gm. extractive.	Sample B. Gm. extractive.	Average per cent.	Description.
Pet. ether	6.0225	5.7879	7.38	Light brown oil
Ether	1.8462	2.0115	2.41	Light brown, resin like
Abs. alc.	13.9224	12.7984	16.68	Dark brown powder
Water	3.7981	3.9932	4.87	Dark brown powder
Dil. alkali	3.8971	4.2131	5.07	Dark brown powder
Dil. acid	2.9317	3.1169	3.77	Med. brown powder
	<hr/> 32.4180	<hr/> 31.9210	<hr/> 40.18	

A larger quantity of the petroleum-ether extractive was desired for further investigation. Accordingly, eleven pounds of the ground fruit were extracted with this solvent. The petroleum was removed by distillation with reduced pressure. The constants of the oil thus obtained were determined as follows: Very bitter taste; bright yellow color; specific gravity 0.9218, at 25°; iodine value (Hanus), 73.1; saponification value, 188.3; acid value 3.5; unsaponifiable material, 1.1%.

SAPONIFICATION OF THE OIL.

Sixty-five Gm. of the oil were saponified with an alcoholic solution of sodium hydroxide, by refluxing for one hour. The alcohol was removed by evaporation; the soap was dissolved in water and extracted with ether (unsaponifiable matter); 50.5 Gm. of fatty acids were obtained when the soap solution was acidulated with diluted hydrochloric acid (1:1). (The presence of the glycerin in the aqueous portion was established by the acrolein test.)

THE FATTY ACIDS.

The separation of the solid acids from the liquid acids was next attempted using the Gusserow-Varrentrapp¹ method. By this method 19.5 Gm. of the liquid-unsaturated acids were obtained.

The bromine addition products² of the unsaturated were now prepared and separated.

The presence of linolenic acid was indicated by the melting point of the small amount of precipitate from the ether solution. A melting point of 182° was observed while the melting point of linolenic acid hexabromide³ is 180–181°. The 4.5 Gm. of precipitate from the petroleum ether, upon recrystallization from alcohol, had a melting point of 111.5°. The melting point of linoleic acid tetrabromide, obtained at this point, is 113–114°.⁴ A bromine determination by the Stepanow⁵ method of this compound showed a bromine content of 53.8 per cent as compared to the calculated 53.33 per cent.

The residue obtained upon the evaporation of the petroleum ether-soluble bromination was a thick paste. A bromine determination, as above, showed it to contain 34.99 per cent as compared to a calculated 36.18 per cent for oleic acid dibromide.

The ether-insoluble lead soap was decomposed with diluted hydrochloric acid. The solid fatty acids floated on the aqueous solution, thus being readily

¹ Lewkowitsch, "Tech. Oils, Fats and Waxes," 6th Edition (1921), page 581.

² *Ibid.*, page 580; ^{3,4} *Ibid.*

⁵ Kamm, "Qual. Org. Anal.," page 168.

removed. By fractional crystallization from alcohol, four portions were obtained. The melting point of each fraction was determined as follows: No. 1, m. p. 51–52°; No. 2, m. p. 50°; No. 3, m. p. 49°; No. 4, m. p. 50°. The melting point of myristic acid¹ is 53.8°, that of lauric acid² is 43.6° and that of palmitic acid³ is 62.62°. Nothing definite can be stated about the identification of the above solid acids. Myristic acid would be indicated with probably lauric or palmitic acid.

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SUGGESTED ASSAYS FOR SOME N. F. PREPARATIONS.*

5. PHENOLATED WATER.

BY J. C. BAUER.

Phenolated Water, N. F. V is wholly dependent upon the phenol which it contains for its therapeutic efficacy; and, as the required phenol content is low (approximately 2 Gm. of phenol to 100 cc. of finished product), it is essential that the full amount be present. The following assay for phenol is, therefore, recommended. It is essentially the method of the United States Pharmacopœia, X, for Phenol *per se* and for Liquefied Phenol.

- Reagents.*—1. 0.1 *N* Bromine solution (Koppeschaar's Solution, U. S. P. X)
2. 0.1 *N* Sodium thiosulphate solution, U. S. P. X
3. Hydrochloric acid, U. S. P. X
4. 20% Potassium iodide solution
5. Chloroform
6. Starch test solution, U. S. P. X.

Procedure.—Dilute 10 cc. of Phenolated Water to 100 cc. Introduce into a 250-cc. glass-stoppered Ehrlenmeyer flask 10 cc. of the diluted sample containing about 0.0206 Gm. of phenol. Add to the contents of the flask and likewise to the contents of a control flask prepared in the same way but containing none of the sample, 25 cc. of 0.1 *N* bromine. Quickly introduce into each flask 5 cc. of hydrochloric acid, insert the stopper and shake during half an hour. Add to each flask as quickly as possible, 5 cc. of potassium iodide solution (20%) and immediately replace the stoppers to prevent the escape of any bromine vapor. Shake well and add to each flask 1 cc. of chloroform to dissolve the precipitated tribromophenol. Titrate the liberated iodine with 0.1 *N* sodium thiosulphate, using starch T.S. as the indicator. The 0.1 *N* bromine remaining in the flask containing the sample subtracted from that remaining in the control flask represents the bromine consumed in the formation of tribromophenol. Each cc. of 0.1 *N* bromine consumed corresponds to 0.001568 Gm. of C₆H₅OH.

Suggested Standard.—Phenolated water contains in each 100 cc., not less than 2 Gm. and not more than 2.2 Gm. of C₆H₅OH.

¹ Lewkowitsch, 6th Edition, page 158.

² *Ibid.*, page 160. ³ *Ibid.*, page 162.

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