

the fires of life burn brightly—the belladonna is in its glory. Output and income of energy are at their height, in preparation for the seasons to follow.

In midsummer, flowers, green fruit and ripe fruit appear, scattered over the belladonna plant. When the plant stands in flower, its alkaloidal principles are formed, in the greater measure in the leaf.

Autumn is the time of ripening and preparation for the future. Growth slackens and stops; starch, sugar and alkaloid are transported to the root; berries ripen, dry and fall to the earth. The intense activity in stem and branch wanes; leaves and stem surrender their living matter to the plant which bore them.

The green color recedes, fades to brown and yellow, the structure dies and falls to the ground, is seized by bacteria and earth worms, to be made over into nourishing soil for the new plant which is to come. Seed and root are covered by the ashes of the dead top, in preparation for the renewed life of another season.

Winter is low tide in plant life. Activity slackens but does not cease. Within root and leaf slowly stored substances are changed. The embryo in the seed takes on shape, buds form on the root crown. Winter is likewise a time of winnowing, sifting and elimination. Weaklings in seeds and roots succumb, rot and are returned to the elements. The few that survive, among the many that perish, take on vigor for the coming season's eternal cycle.

ISOLATION OF THE OIL AND ALKALOIDS OF STAVESACRE SEED.

(Delphinium Staphisagria).

BY L. N. MARKWOOD.*

Following the investigation on *Delphinium consolida* (13) investigation of the seed of *D. staphisagria*, commonly known as stavesacre, was undertaken to isolate in pure form the oil and alkaloids in order to determine their insecticidal activity.

In the past century various reports of work on the alkaloids of the genus *Delphinium* mainly *D. staphisagria*, have been made. Among these the outstanding contributions have been made by Marquis (14), whose formulas for delphinine, $C_{22}H_{33}NO_{63}$, m. p. "above 120° ," and the three other alkaloids he claims to have isolated have been widely copied in textbooks; by Kara-Stojanow (9), who ascribed to delphinine the formula $C_{31}H_{49}NO_7$, m. p. 191.8° (cor.); by Katz (10), who reported no formula or melting point but undoubtedly had the pure alkaloid and performed the first experiments on chemical structure; by Walz (17), who, having at his disposal 50 Gm. of crystallized delphinine (Merck), established the formula $C_{34}H_{47}NO_9$, m. p. 187.5° (uncor.); and by Keller (11), who made no determination of the formula but found the m. p. 187.5° (uncor.) and performed experiments on the constitution.

Since a large part of the success in obtaining pure alkaloids depends on the solvent for extraction, tests were made to determine the suitability of various solvents. Previous investigators had used as solvents alcohol, alcohol with acetic

* Assistant Chemist, Food, Drug and Insecticide Administration, U. S. Department of Agriculture, Washington, D. C.

or tartaric acids, ether and petroleum ether (to remove fatty matter) followed by alcohol. It was found by trial that petroleum ether alone removes the oil completely, extracts the alkaloids to a satisfactory degree, and at the same time carries along a minimum of impurity. That this direct solubility of the alkaloids in the organic solvent proves their occurrence as free bases is not to be taken for granted, as the oil is a factor in the solubility.

The seed were first stripped of their heavy seed-coats (2) which contain some extractable matter, especially coloring matter, but no alkaloids. To facilitate stripping the seed were softened by immersion in water for 1 to 2 hours. The water was drained off, and the seed were then decorticated in a machine designed by the writer especially for this purpose. The machine consisted essentially of a rotatable wooden cone surfaced with tin grater metal, fitted loosely inside a similarly surfaced hollow block. As the seed fell between the rotating cone and the stationary block the seed coats were pulled off. The material was then dried, and the seedcoats were removed by means of an air separator.

The kernels were coarsely ground in a mill, then washed with petroleum ether to remove expressed oil, and reground to a fine powder, approximately No. 60. This powder was packed in a percolator and extracted with petroleum ether. The powder must be very fine to secure a satisfactory measure of extraction, as the petroleum ether lacks great penetrating power. The percolation was stopped when a test showed practically no oil in several drops of percolate.

The solvent was distilled from the percolate by heating on the water-bath at ordinary pressure. The residue, a brown oil, was dissolved in twice its volume of ethyl ether preparatory to removal of the alkaloids by extraction with sulphuric acid.

Soon after the ether was added a white flocculent substance was deposited on the bottom of the vessel. After the solution had stood over night to permit complete precipitation the deposit was filtered off. This deposit showed double refraction, indicating a crystalline structure. It was insoluble in ether, petroleum ether and alcohol, but dissolved when warmed in the pure oil of stavesacre or in the ether-oil solution. It was redeposited on cooling. On ignition it emitted the odor of burning fat and left a residue containing calcium, as shown by the flame test. It, therefore, appeared to be a calcium-fatty acid compound. The free fatty acid was found to be non-volatile and was readily soluble in ether. After repeated recrystallizations from alcohol it melted at 55-56°. The ethyl ester melted at 17.6°. These data indicate that it may be the C 17 acid of the acetic acid series, known variously as daturic or margaric acid, or simply as heptadecylic acid. However, owing to the fact that the occurrence of odd-carbon fatty acids in nature is a subject of keen dispute (6) this conclusion is presented only tentatively, and more work is needed to settle the point. Fresh U. S. P. ether was slow in causing precipitation of the calcium compound from the ether-oil solution, whereas ether that had been used previously and recovered brought about its precipitation almost immediately. The only difference ascertained between the two samples of ether, to which this behavior might be traced, was the presence of water, approximately to the point of saturation, in the used ether and its relative absence in the fresh ether. It is suggested that the water reduced the solubility of the calcium compound in the oil.

The ether solution containing the oil and alkaloids was extracted with portions of 5 per cent sulphuric acid until tests showed complete removal of the alkaloids. The ether solution was freed from acid by thorough washing with water, after which the ether was distilled on the water-bath. To expel the last traces of solvent the oil was heated to 120° under reduced pressure.

Analysis of the oil:¹

Specific gravity 20/4	0.891
Refractive index at 20°	1.462
Acid value	92.8
Iodine no. (Hanus)	80.2
Saponification value	190.0
Acetyl value	12.8
Unsaponifiable	2.92 per cent

The sulphuric acid solution containing the alkaloids was a deep brown and slightly turbid. It was washed with chloroform, which removed an appreciable amount of color but at the same time dissolved out some alkaloids. On account of the impurity of the chloroform solution nothing of value could be recovered from it.

The aqueous acid solution was freed from dissolved chloroform by warming to 60° under vacuum. To it was added sodium hydroxide solution in slight excess, causing precipitation of the alkaloids as a curdy, bulky mass tinged yellow by absorption of coloring matter. The precipitate was filtered and washed. More precipitate was obtained by adding more sodium hydroxide to the filtrate.

The precipitate was dissolved in the minimum amount of dilute sulphuric acid. On standing some crystals of calcium sulphate developed, showing that calcium had been removed from the oil by the acid. A second precipitation with sodium hydroxide brought down the alkaloids colored yellow as before. The precipitate was filtered and washed with water as before, sucked as dry as possible, and dissolved in alcohol. The alcohol solution on standing deposited more calcium sulfate this time in gelatinous or amorphous form, as well as some potassium sulfate. The potassium also was derived from the oil.

After the alcohol was evaporated the alkaloid residue was dissolved in a small quantity of chloroform, forming a brown solution. The addition to this solution of five times its volume of ether resulted in a clear slightly yellow ether solution and a viscous almost resinous deposit.

The ether solution was poured off and to it was added several Gm. of crushed silica gel. The mixture was slowly evaporated, treated with more ether and again evaporated. It was then taken up in ether and filtered free from the silica gel and its adsorbed impurities. On evaporation of the ether, crystals of the alkaloid appeared.

An attempt to obtain crystals from the ether-insoluble fraction dissolved in alcohol by treatment with silica gel was unsuccessful.

The filtrate from the precipitation with sodium hydroxide, which contained appreciable quantities of alkaloid, was extracted with chloroform. After evapora-

¹ Conducted by W. F. Baughman of the Oil, Fat and Wax Laboratory, Bureau of Chemistry and Soils.

tion of the cholroform the residue was separated into ether-soluble and ether-insoluble fractions. Only the ether-soluble fraction yielded crystals.

The combined crystal residues were recrystallized from alcohol twice. The crystals, which appeared as 6-sided colorless plates had a melting point (uncor.) of 187.5-187.8°.

Analysis of the crystals resulted as follows:¹

0.1207 Gm. gave 0.2948 Gm. CO₂ and 0.0837 Gm. H₂O. Carbon 66.60%. Hydrogen 7.76%. 0.0503 Gm. consumed 4.15 cc. of 0.01948 N HCl. Nitrogen 2.25%.

	C.	H.	N.
Calculated for C ₃₄ H ₄₇ NO ₉	66.52	7.72	2.28
Found	66.60	7.76	2.25

This formula and the melting point above agree with the values found by Walz (17), and identify the alkaloid as delphinine.

A crystallographic study of this alkaloid was undertaken by C. M. Smith of the Insecticide, Fungicide and Caustic Poison Control. His report follows:

Delphinine has been examined previously by Lagorio (14) and by Arndt (9). Their results, however, did not entirely agree. Measurement of the crystals prepared in this investigation fully corroborate Arndt's figures. See Table I.

TABLE I.

System.	Axial ratio.	Forms				Interfacial angles.				
		Basal pina- coid c(001).	Brachy- pina- coid b(010).	Unit pyramid o(111).	Unit macro- dome r(101).	r:r'.	o:o'.	r:c.	o:c.	
Lagorio	Orthorhombic	0.637:1:0.804	Present	Present	Present	Absent
Arndt	Orthorhombic	0.783:1:1.017	Present	Present	Present	Present	104°48'	117°33'	127°39'	121°28'
Smith	Orthorhombic	0.77:1:1.01	Present	Present	Present	Present	104°57'	117°21'	127°33'	121°10'

Further examination of the crystals by means of the polarizing microscope showed the characteristics of the orthorhombic system. The average index of refraction is 1.56, and the maximum birefringence approximately 0.006. Lagorio's statement that the birefringence is positive could neither be confirmed nor disproved, owing to the indistinct interference figures obtained.

No evidence of other crystallizable bases was found, and, although several other alkaloids both crystalline and amorphous have been reported in the literature, delphinine remains the only one that has been consistently obtained.

SUMMARY.

Stavesacre seed by extraction with petroleum ether yielded a fixed oil, the properties of which have been determined. It yielded also the alkaloid, delphinine, previously known.

REFERENCES.

- (1) F. B. Ahrens, "Staphisagroin ein neues Alkaloid," *Berichte*, 32, 1581-4 (1899).
- (2) C. W. Ballard, "Microscopy of Larkspur and Stavesacre," *PROCEEDINGS A. PH. A.* 57, 892-6 (1909).
- (3) R. Boehm, "Beiträge zur Kenntniss der Alkaloide der Stephanskörner," *Arch. expl. Path. und Pharmakol.*, 5, 311-28 (1876).

¹ Determination of carbon and hydrogen made by Mr. R. M. Hann of the Bureau of Chemistry and Soils. Determination of nitrogen made by Mrs. M. K. Murray of the Fixed Nitrogen Laboratory.

- (4) R. Brandes, "Ueber das Delphinin," *Schweigger's J. f. Chem. u. Phys.*, 25, 369-78 (1819).
- (5) J. P. Couerbe, "De la delphine," *Ann. Chim. Phys.*, (2) 52, 359-68 (1833). Also in *Ann. der Chem.*, 9, 101-8 (1834).
- (6) H. Dieterle, "Datura alba Nees," *Arch. Pharm.*, 264, 140-64 (1926).
- (7) J. Erdmann, "Ueber Delphinin," *Arch. Pharm.*, 117, 43-6 (1864).
- (8) M. O. Henry, "Sur la delphine et la solanine," *J. Pharm.*, 18, 661-6 (1832).
- (9) C. Kara-Stojanow, "Ueber die Alkaloide des *D. staphisagria*," *Pharm. Zeitsch. f. Russland*, 29, 628-30, 641-8, 657-64, 673-8, 689-92, 705-8, 721-6 (1890). This article is abstracted in *J. Chem. Soc. (Br.)*, 60, 842-3 (1891).
- (10) J. Katz, "Ueber die Alkaloide des *D. staphisagria*," *Pharm. Ztg.*, 735-6 (1900).
- (11) O. Keller, "Untersuchungen über die Gruppe der Helleboreen," *Arch. Pharm.*, 263, 274-93 (1925).
- (12) J. L. Lassaigne and H. Feneulle, "Analyse de la staphisaigre," *Ann. Chim. Phys.*, 12, 358-71 (1819).
- (13) L. N. Markwood, "The Isolation and Properties of the Alkaloids and Oil of Larkspur Seed (*Delphinium consolida*)," THIS JOURNAL, 13, 696-702 (1924).
- (14) Marquis, "Ueber die Alkaloide des *D. staphisagria*," *Arch. exptl. Path. und Pharmakol.* 7, 55-80 (1877). Also in *Pharm. Zeitsch. f. Russland*, 16, 449-59, 481-92, 513-22 (1877). Abstracted in *Jahresbericht*, 894-897 (1877).
- (15) J. Serck, "Beitrag zur Kenntniss des Delphinins," Dissertation, Dorpat (1874.)
- (16) B. Studer, "Ueber die Alkaloide der *Staphisagria*," *Schweiz. Wochenschrift f. Pharm.* 10, 132-4 (1872). Abstracted in *Jahresbericht über die Fortschritte der Pharmacognosie*, 7, 148 50 (1872).
- (17) Thomas Walz, "Delphinin," *Arch. Pharm.*, 260, 9-26 (1922).

CHLORINATION OF ANTIPYRINE. I. CHLOROANTIPYRINE.*

BY W. O. EMERY.

While the apparent ease with which antipyrine responds to the action of chlorinating agents has been noted by several investigators, its behavior in this respect has not apparently been accorded the importance it merits. Schweisinger¹ observed the formation of a white precipitate on treating antipyrine with an aqueous solution of calcium hypochlorite. Gay and Fortune,² on the other hand, found that this reagent is without effect on antipyrine in the cold, but does yield on boiling a yellow solution. Berenger³ reported that chlorine water, as also sodium hypochlorite solution, loses in contact with antipyrine its characteristic odor, yielding with the former a white precipitate. Michaelis and co-workers (Röhmer and Pasternack resp.)⁴ prepared by the action of phosphoroychloride on antipyrine the dichloro substitution product, $C_{11}H_{12}N_2Cl_2$, from which alkali readily regenerates the mother substance. In a study of the behavior of iodo- and bromoantipyrine toward potassium iodide and hydrochloric acid, Bougault⁵ mentioned a chloroantipyrine corresponding apparently with the composition, $C_{11}H_{11}N_2ClO$,

* Contribution from Special Collaborative Investigations, Bureau of Chemistry, U. S. Dept. of Agriculture.

¹ *J. Pharm. Chim.* (V), 11, 31 (1885).

² *Ibid.*, (V), 17, 596 (1888).

³ *Ibid.*, (V), 18, 393 (1903).

⁴ *Ber.*, 31, 3194 (1898); 32, 2398 (1899).

⁵ *J. Pharm. Chim.* (VII), 20, 248 (1919).