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THE CHEMISTRY OF THE VOLATILE OIL OF MILFOIL.*

A Study of the Application of Modern Organic Chemistry to Drug Plant Investigations.† **

BY ROLAND E. KREMERS.

In recent years the experimental culture of drug plants on a semi-commercial scale has reëmphasized the fact that ultimate success depends upon the rational application of the modern sciences, and in particular has opened an attractive field for applied chemistry. Plant chemistry, or phytochemistry, as it is sometimes called, is a composite study, requiring in its entirety a knowledge not merely of chemistry, but of botany, pharmacy, pharmacology, physiography, climatology, soils, and many other phases of science. Since no individual can devote special attention to all of these relations it is the more necessary for those who study the various phases of the subject to explain to their associates their methods and results. Hence it has seemed more rational to present the recent researches on the oil of milfoil from the point of view of the organic chemist, because that has been the training of the writer.

Although milfoil, botanically—Achillea millefolium—was known to Dioscorides[1] and has enjoyed a varying popularity as a remedy through all ages, and in spite of the fact that the first chemical examination was made by Bley[2] as far back as 1828, Miller[3] wrote in 1916 that up to that time only two constituents had been definitely identified—acetic acid and cineol. He himself, however, by a very painstaking investigation, carried out while chemist of the Wisconsin Pharmaceutical Experiment Station, was able to add very materially to the list of known constituents of this oil; and the work of the present investigator has been in continuation of his. As it is not so much the object of this paper to de-

[1] Dioscorides, Materia Medica, cf. Miller, p. 5.

[2] L. F. Bley, *Trommsdorf's Neues Jour. d. Pharm.*, 16, I, pp. 245–73 (1828); and *Ibid.*, 16, II, pp. 94–120 (1828); cf. Miller, p. 7.

[3] E. R. Miller, "The Chem. of the Oil of Milfoil," *Bull.* 785, Univ. of Wis.

scribe the minute details of the work of the past five years as to show how the viewpoint of the organic chemist is expressed in the results thereof, the steps of Professor Miller's work will be omitted.

After the organic chemist has obtained his materials, either by synthesis, or from the trade, or as by-products, he must establish their identity and purity. In a like manner, the plant chemist must concern himself about the authenticity of the botanical material, and its freedom from contamination or adulteration, either accidental or wilful. In the present instance, *Achillea millefolium* is the only common representative of the genus in the vicinity of Madison, Wisconsin, where the herb was collected. The purity of the product was secured by carefully picking over by hand which eliminated grass, weeds, dirt and other foreign matter. In the case of plant materials, a further precaution is almost always necessary. Either the oil must be distilled immediately, or the crude drug must be appropriately handled to prevent biochemical change. The herb gathered in the summer of 1919 was immediately spread out in a thin layer on the attic floor of the chemistry building, where it dried rapidly. With the process of desiccation some oil was undoubtedly lost, but, as Prof. Miller found, no unusual variation occurs in either the percent yields or the physical constants of oils from both fresh and dry herb, and the enzyme or ferment action is undoubtedly largely checked by drying.

The collection of the herb, though by no means a continuous occupation, lasted nearly a month, or from the middle of June to the middle of July. The distillation of the dried herb was accomplished in a comparatively short campaign of three days. A total fresh weight of about 1200 pounds of herb was collected; dried, this weighed 420.5 pounds, or 190.7 Kg., and yielded 892 Gm. of oil, or 0.467 p. c. of the dry weight.

Another consideration of the organic chemist is that of obtaining a maximum yield. The question of the various methods of distillation and their results will not be discussed, partly for the reason that only the ordinary simple steam stills were available. But even with this apparatus, it was possible to increase the yield of oil. A moment's consideration shows that the distillate consisting of the two phase liquid system, oil and water, is produced from the single phase system, steam and oil vapor. Hence, because of the intimate mixture of the gaseous system, the liquid phases are in effect saturated solutions. The quantity of oil held in the water is still further increased by the extreme subdivision of some of the droplets which do not separate even on several days' standing. The ordinary practice is to separate the oil, by passing the distillate through a Florentine flask or sometimes several of them. The water is then discarded. For this investigation, however, the latter was collected and submitted to the process of cohobation. That is, it was distilled from a steam-jacketed still until about one-fourth to one-third had passed over; the residue was discarded and the distillate subjected a second and a third time to this process, at the end of which time the volume had been reduced to about 4 liters. Each cohobation yielded more oil. The first 105 Gm.; the second and third together, 94 Gm. Thus an additional 199 Gm. of oil were obtained, or an amount equal to 21 p. c. of the yield of the original oil.

The materials, then, obtained from the herb were, for this investigation first, the original separation of oil; second, the cohobated oils; and third, the

cohobated aqueous distillate. The investigation of these was taken up in the reverse order as a matter of convenience.

When confronted with the problem of separating and identifying the components of a mixture, the organic chemist has recourse to either physical or chemical means. Frequently a combination of the two methods of resolution are employed, as in this instance. Because of the great similarities exhibited by such closely related compounds as the terpenes, no simple scheme of analysis is applicable, and it is necessary to resort to a detailed study of reactions and derivatives. In the following paragraphs brief mention is made of the means used to isolate, approximately, the various constituents of the above materials, and of the reactions or derivatives used to characterize further the compounds whose presence had been indicated.

EXAMINATION OF THE AQUEOUS DISTILLATE.

The cohobated aqueous distillate was subjected to fractional distillation as the most likely way of determining the substances which were present. After repeated fractionations, the following series of fractions resulted:

1st................	up to 64° C.	5 Cc.
2nd...............	64° to 66°	68 Cc.
3rd...............	66° to 70°	55 Cc.
4th...............	70° to 75°	22 Cc.
5th...............	75° to 80°	3 Cc.
6th...............	80° to 90°	5 Cc.
7th...............	90° to 100°	..
8th..............	Oil separation by distillation.	

The boiling point and odor indicated that methyl alcohol was the chief constituent. A chemical examination of these fractions led to the following results:

H_2CO— Formaldehyde.—The first runnings of the final cohobation possessed a sharp formaldehyde odor and gave an instantaneous reaction with Schiff's reagent. The resorcinol test was not quite typical, however.

CH_3OH— Methyl alcohol.—From fraction 64–66° methyl salicylate, formaldehyde, and 3-, 5-dinitrobenzoic methyl ester (m. p. 108°) were obtained.

C_2H_5OH— Ethyl alcohol.—Iodoform reaction from frac. b. p. 70–75°.

C_3H_6O— Acetone.—Reaction with Na-nitroprusside and with I-KI from fraction —64°.

$C_5H_4O_2$— Furfural.—From fraction 90–100° and from separated oil by aniline acetate reaction.

$C_{10}H_{18}O$— Borneol.—Crystallized from oil, m. p. 203–4° C.

The oil contained some acids and some aldehydes besides borneol and other constituents. The quantity was so small, however, that the material was added to the cohobated oils.

PHYSICAL AND CHEMICAL CONSTANTS OF THE OILS.

Just as the organic chemicals are judged as to purity by their constants, so the volatile oils are judged by the study of appropriate determinations. However, as the latter are almost invariably mixtures, and often complex mixtures, these "constants" vary. In the present instance, the purity was beyond question, hence the following determinations were made to help establish the limits of variation, as well as to throw a little light on the presence of certain types of compounds.

The following constants were recorded:

	1st oil.		Cohobated oils.		
	1916.	1919.	1916.		1919.
Constant.				1st.	2nd and 3rd.
Density at 17 = ...	0.915	0.913	at 25 = 0.939	0.948	0.959
Acid No................	7.24	4.27	37.0		
Ester No................	2.2	5.65	7.6	*Av.*, 17.7	
Sap. No................	9.44	10.92	44.6		
Ester No. after acetyl....	23.5	17.8	51.8	*Av.*, 50.9	

From the above, it is evident that the cohobated oils are appreciably more dense, that they contain more free acid, more ester, and more free alcohols. This is what might be expected from the consideration that the oil and water of the original distillation are two mutually saturated solutions, because these substances are more soluble in water than the hydrocarbons.

CONSTITUENTS OF THE COHOBATED OILS.

The combined 1916 and 1919 oils were first subjected to further investigation because they were of a convenient bulk. The acid was removed with Na_2CO_3 solution; the aldehydes by $NaHSO_3$ solution; and the esters were saponified by alcoholic NaOH V. S. The acids were regenerated by the addition of an excess of 50 p. c. H_2SO_4 solution and distilled over with steam. Part of the material was converted into the sodium salts and then into silver salts. The aldehydes or ketones were regenerated by the addition of sodium hydroxide and extracted with ether. Their conversion into characteristic derivatives has not been successful so far.

Two preparations of Ag salts gave the following p. c. of Ag.

A 1.............. 51.22 p. c.   B 1.............. 50.66 p. c.
  2.............. 53.47 p. c.   2.............. 51.57 p. c.
                                     3.............. 52.43 p. c.
                                     4.............. 52.88 p. c.

Also, a precipitate of high Ag content was formed from the mother liquors on warming, indicating a formate. A more soluble salt has not as yet been characterized.

$C_4H_7O_2Ag$........................ = 55.23 p. c. Ag
$C_5H_9O_2Ag$........................ = 51.67 p. c. Ag
$C_6H_{11}O_2Ag$........................ = 48.43 p. c. Ag

A valeric acid is, therefore, probably present.

The regenerated aldehydes gave a positive test for furfural by the aniline acetate reaction. A preliminary test also seemed to yield a crystalline phenyl-hydrazone, but when it was prepared in a larger quantity to determine the melting point, all efforts to effect crystallization were futile.

The neutral oils were saponified as usual and then subjected to fractional distillation. So far, no other result has been obtained than the separation of a large quantity of impure borneol.

The ether extract of the alkaline saponification residue yielded a trace of eugenol. Benzoyl ester m. p. 69–70°.

CONSTITUENTS OF OIL (FIRST SEPARATION).

In order to avoid possible change in the constituents of the oil due to re-agents, fractionation was resorted to directly and no attempt was made to isolate

constituents by chemical means. After repeated fractionations, in part under atmospheric pressure, in part under a vacuum of 30 mm., the following series was obtained:

Frac. No.	B. p.	Vol. ψ.	d_{28}.	n.	α.
1........................	$-85°$ C.	10 Cc.	...	....	...
2........................	85–155	30	...	....	...
3........................	155– 60	320	0.850	1.4655	—16.1°
4........................	160– 61	170	0.852	1.4670	—14.4
5........................	161– 62	160	0.852	1.4675	—12.9
6........................	162– 63	130	0.853	1.4675	—10.75
7........................	163– 64	110	0.855	1.4688	— 9.37
8........................	164– 66	170	0.855	1.4684	— 8.03
9........................	166– 70	210	0.862	1.4692	— 5.07
10........................	170– 74	50	0.871	1.4705	— 2.50
11........................	75– 80	260	0.871	1.4675	— 4.44 at 30 mm.
12........................	80– 85	250	0.881	1.4666	— 1.84
13........................	85– 90	90	0.890	1.4655	— 1.56
14........................	90– 95	150	0.908	1.4618	— 4.78
15........................	95–100	80	0.920	1.4608	— 6.94
16........................	100– 05	300	0.930	1.4618	—12.75
17........................	105– 10	110	0.940	1.4642	—17.65
18........................	110– 15	190	0.949	1.4726	—24.3
19........................	115– 20	50	0.949	1.4700	—23.4
20........................	120– 25	25	0.957	1.4750	—28.4
21........................	125	residue	...	....	...

From the material corresponding to these fractions, Prof. Miller obtained the following compounds:

A valeric acid.—This was found free in the oil and was isolated by shaking out various fractions with a sodium carbonate solution.

Salicylic acid.—This was shaken out by means of sodium hydroxide solution.

Butyric acid was found in the saponification residue.

Aldehydes were shown to be present by qualitative reactions. Their amount was undoubtedly minute.

The *pinene* fraction yielded the pinene nitroso-chloride, m. p. 102–3° C.

Limonene was indicated by the gravity of the appropriate fractions, but no nitroso-chloride was obtained.

Camphor was separated from borneol by the phthalic acid anhydride method and converted into two characteristic derivatives, the semicarbazone, m. p. 235–6°, and the oxime, m. p. 118°.

Borneol was obtained in a state of purity, m. p. 202–3°. Two derivatives were prepared in addition—the acetate, m. p. 29° C., and the phenylurethane, m. p. 139° C.

Cineol after being isolated by the resorcinol method was converted into the iodol addition product. This was found to melt at 106–7° instead of at 111–12° C.

The present investigation confirmed the presence of these compounds in so far as they were investigated, namely, pinene, cineol, and the borneol camphor mixture. The hypothetical presence of limonene was neither proved nor disproved, though the possible presence of cymene seems to be excluded by spectrographic measurements. In addition the presence of nopinene was established by Wallach's permanganate oxidation. M. p. of nopinic acid, 126° C., and of nopinone semicarbazone, 188° C.

As a further guide to the composition of the higher fractions, the quantitative saponification was undertaken both before and after acetylation. The results are as follows:

Frac. b. p.	Ester No.	P. c. ester.	E. N. after acetyl.	Diff.	P. c. alcohol.
90– 95°	11.3	3.9	29.5	18.2	5.0
95–100°	12.5	4.2	33.7	21.2	5.8
100–105°	20.5	7.2	50.9	30.4	8.3
105–110°	17.3	6.0	87.5	70.2	19.3
110–115°	50.3	17.5	177	126.7	34.9
115–120°	38.6	13.3	136	97.4	26.7
120–125°	89.2	31.2	202	110.8	30.5
125° +	50.2	17.5	115	64.8	17.8

Because of the presence of such an appreciable quantity of ester in the higher fractions, it was desirable to saponify before further examination. This was done in the usual manner. The saponified fractions were then several times refractionated, yielding the following series:

B. p. 15 mm.	Vol. cc.	d_{25}.	n_{25}.	α.	
65°	...	0.863	1.4682	— 9.4°	
65– 70°	164	0.866	1.4700	— 5.8°	
70– 75°	200	0.872	1.4715	— 1.07°	
75– 80°	75	0.882	1.4700	+ 1.5°	
80– 85°	40	0.892	1.4650	+ 3.0°	
85– 90°	100	0.907	1.4600	+ 3.6°	
90– 95°	330	0.929	1.4588	— 3.0°	
95–100°	115	0.938	1.4656	—13.5°	
100–105°	77	0.946	1.4720	—20.5°	
105–110°	110	0.950	1.4765	...	
110–120°	50	0.950	1.4815	...	
*120–130°	50	...	....	...	separated borneol
130–140°	40	...	colored green	...	
140–170°	55	...	dark blue	...	
170–195°	35	...	....	...	

* These fractions do not represent the true proportion of the higher boiling constituents which were largely set aside after the preliminary fractionations to be separately investigated for azulene.

The constants for fraction 90–95° suggested the presence of thujone, which was found upon examination. The tribromide prepared according to Wallach's method melted at 121°. The semicarbazone was readily formed, but was either a mixture of the isomers α- and β-thujone, or thujone with another ketone. A preliminary experiment also showed the presence of a ketone capable of slowly combining with NH_4HSO_3. Hence there seems no reasonable ground to doubt the presence of thujone.

EXAMINATION OF HIGH BOILING FRACTIONS AND ISOLATION OF BLUE HYDROCARBON.

The residues b. p. 120°+ of the 1915 and 1916 oils were fractionated into the following:

1 B. p................................ 120°
2 B. p................................ 120–40°
3 B. p................................ 140°+

The residues b. p. 140° + were then worked up for the blue hydrocarbon. Sherndal had shown that it could be isolated by means of H_3PO_4. Hence his method was employed, though modified by using 4 volumes of heptane as a diluent of the oil. In the case of a typical experiment 100 Gm. of the fraction b. p. 140°+ yielded 35.5 Gm. of regenerated blue oil and 59 Gm. of non-blue oil. In all a

considerable quantity of azulene was obtained, sufficient for the determination of a few physical constants. The boiling point was determined in connection with the distillation which served as the final purification. The carefully regulated operation showed a constant b. p. 135–36° at 1.1 mm.; bath temperature = 175° C.

No determinations of the index of refraction or of the optical activity were possible because of the intensity of the color. This material yielded the picric acid addition compound instantly with an alcoholic solution of picric acid.

Reduction and oxidation experiments as well as the measuring of the absorption bands have not been completed up to the present time and will be made the subject of a separate report.

The oil after removal of the azulene by means of H_3PO_4 was fractionated under diminished pressure. After several runs the results were as follows:

	B. p.	Pressure.	Amount. Gm.	$d_{16}°$.	n_{16}.
1.............	—80°	at 5 mm.	27	...	1.4240
2.............	80–100°	2	45.5	0.947	1.4800
3.............	100–110°	0.5	68	0.930	1.4940
4.............	110– 15°	0.5	28	0.923	1.4995
5.............	115– 20°	0.5	7	...	1.4982
6.............	120– 40°	0.1	43	0.953	1.4975
7.............	140– 60°	0.1	29	...	1.5000
8.............	160– 80°	0.1	..	...	1.4963

The fractions between 100° and 115° showed close approximation to the constants of the sesquiterpenes. They were distilled over Na and yielded a fraction:

B. P. 105–10°   30 Gm. d_{20} = 0.916   n = 1.4990   M. R. = 65.4

α = —13.75° for 100 mm. column of oil,—

from which the following derivatives of caryophyllene were obtained:

Benzylamine base, M. p. 172–3° C.,

Caryophyllene hydrate, M. p. 94–5°,

No nitrosite.

SUMMARY OF COMPOUNDS ISOLATED.

The present investigation isolated or confirmed the following constituents:

Methyl alcohol, formaldehyde, probably formic acid, ethyl alcohol, acetone, furfural, valeric acid, eugenol, pinene, nopinene, cineol, thujone, borneol, camphor, caryophyllene, and azulene. The following were found by Prof. Miller, but not tested for in the present investigation up to date: acetic acid, butyric acid, salicylic acid, a high boiling acid or lactone.

DISCUSSION OF RESULTS.

One of the first questions confronting an organic chemist in the study of any compound or series of compounds, is that of classification—or logically—where does this or that compound belong? Following a rational system of classification according to

1. Hydrocarbon, or class of substitution products
2. The degree of saturation
3. Chain or cyclic character
4. No. of C atoms in the molecule.

The results of the investigations on milfoil oils to date may be classified as follows:

Config-uration.	Hydro-carbons.	Mono–OH subst.	Di–OH subst.	Tri–OH subst.	Tetra–OH subst.
....	......	......	Series C_nH_{2n+2}	......	......
....	......	Methyl alcohol	Formaldehyde	Formic acid	......
....	......	Ethyl alcohol	......	Acetic acid	......
....	......	......	Acetone	......	......
....	......	......	......	Butyric acid	......
....	......	......	......	Isovaleric acid	......
....	......	......	Series C_nH_{2n}	......	......
Δ	......	......	Cineol	......	......
....	......	......	Series C_nH_{2n-2}	......	......
F_2	......	......	......	......	Furfural
$Δ_2$	......	Borneol	Camphor	......	......
....	......	......	Thujone	......	......
....	......	......	Series C_nH_{2n-4}	......	......
F, $Δ_2$	Pinene	......	......	......	......
....	Nopinene	......	......	......	......
....	......	......	Series C_nH_{2n-6}	......	......
F_2, $Δ_2$	Caryophyllene	......	......	......	......
F_3, Δ	......	......	......	......	Salicylic acid
....	......	......	Series C_nH_{2n-8}	......	......
F_4, Δ	......	......	Eugenol	......	......
....	......	......	Series C_nH_{2n-12}	......	......
....	Azulene	......	......	......	......

GENETIC RELATIONSHIPS.

In spite of the fact that the list of substances occurring in the oil of milfoil is still admittedly incomplete, the above classification brings out certain relationships. The trio of compounds, methyl alcohol, formaldehyde, and formic acid, suggests the process of auto-oxidation and reduction:

$$2HCHO \longrightarrow CH_3 - O - CH = O + H_2O \longrightarrow CH_3OH + HCOOH$$
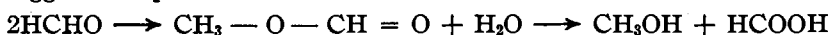
Acetaldehyde has not been isolated, but the analogous reaction products, ethyl alcohol and acetic acid, have been. A reinvestigation should not only make doubly sure of these compounds, but also make a careful search for the aldehyde. A comparable fact is the presence of borneol and camphor. Another ketone, thujone, has been proved to be a constituent; by analogy thujyl alcohol is a possible companion and should be looked for.

The origin of all of the compounds found in a given distillate is not necessarily the same. Furfural is known to be very readily obtained by dehydration of aldo-pentoses, and is usually looked upon as a decomposition product. Accordingly an examination of the non-volatile constituents of *Achillea millefolium* would start with this clue concerning the carbohydrates to be tested for.

In the laboratory it is possible to convert pinene into compounds of the borneol and camphor type. The occurrence of both pinene and borneol in this oil as in so many others suggests that plant metabolism is able to accomplish a similar change. Nopinene is also related by simple reactions to the borneol type.

Of a different order is the relation of azulene, $C_{15}H_{18}$, to the sesquiterpenes and sesquiterpene alcohols, $C_{15}H_{24}$ and $C_{15}H_{25}OH$, respectively. Since the struc-

ture of none of these compounds is understood, any known relation must be largely empirical. The most obvious is the difference of six atoms of hydrogen. Of recent years various investigators have reported the occurrence of blue fractions resulting from the dehydration of sesquiterpene alcohols, or from the oxidation of the sesquiterpenes themselves. Sherndal not only isolated and characterized azulene, but also showed that the color reactions of certain sesquiterpenes were due to the formation of this substance. That there is a genetic relationship between certain compounds $C_{15}H_{24}$ and azulene is unquestionable. But how to account for the loss of six atoms of hydrogen in the plant is still an unsolved problem.

Another problem which is constantly confronting the organic chemist is the one of naming his compounds. The Geneva Congress adopted a number of generalizations which have found all too little response in this country. Like all things human, they have their limitations. For the chain and single cycle types of compounds it does very well with certain modifications. But when it comes to the bi-, or even tri-cyclic compounds such as are common constituents of the volatile oils, the limitations are soon manifest. Furthermore, it seems not irrational to state that, at the same time that these compounds in the volatile oils are the first to show the limitations of Geneva Congress nomenclature, they furnish the point of attack which will give the key to further advances. The reason is that in the groups of compounds known as the terpenes, terpene-alcohols, and camphors, we have a practically complete representation of the possible configurations, from those compounds which have no cycles to those in which the unsaturation is accounted for solely by cycles. We find cycle propane derivatives, cyclo butane, cyclo pentane, cyclo hexane derivatives, simple cycles, and cycles with side-chains. And the *abbau* and *aufbau* products have ramified into practically all the common types of compounds which we know to-day, and some rare ones as well.

And lastly, attention should be directed toward the methods and reactions employed to isolate and identify the constituents of this oil. Physical means are important guides and preliminary steps. Some reactions have not progressed beyond the stage of color tests, as in the case of formaldehyde, furfural, and salicylic acid. Others are based on slightly less empirical data—the percent of Ag in silver salts; the formation of iodoform under certain conditions in the case of acetone and ethyl alcohol. Most convincing, because most in accord with the best principles of modern organic chemistry, are the demonstrations based on the preparation, isolation, and characterization of specific derivatives dependent upon the specific structure and reactions of the compound to be identified. The pinene nitrol piperidine base; caryophyllene nitrol benzylamine base, and caryophyllene hydrate; the azulene picrate; camphor oxime; borneol phenyl urethane; cineol iodol compound and the 3-5-dinitrobenzoic ester of methyl alcohol, are examples of this kind. In certain instances it has been possible to separate the pure substances themselves, as borneol and camphor, because of their great power of crystallization and high melting points.

It is a temptation to conclude by enlarging upon the fascinating problems suggested by those results of an investigation into the composition of the volatile oil of a well-known drug plant. However, it must suffice to mention only the

coöperative studies now in progress. The pharmacological action of azulene is being investigated by Dr. A. S. Loevenhart and his students.[1] In conjunction with Dr. G. L. Clark the study of the surface energy relations has been begun. This research will include a general survey of all of the types of compounds occurring in the volatile oils. Thus does the rational chemical study of a single drug plant lead on and on. What the future will reveal is but speculation to-day. Yet as Dr. F. B. Power[2] has remarked, "it may be noted that one of the largest and most attractive fields of chemical investigation still remains practically unexplored, for comparatively little is yet known respecting the constituents of the plants which inhabit North America."

NOTES ON EMETINE BISMUTH IODIDE.

BY GEO. E. EWE.

Emetine bismuth iodide was brought to the attention of the medical profession for the treatment of entamoebiasis by A. G. DuMez, formerly of School of Pharmacy, College of Medicine and Surgery, University of the Philippines, in an article entitled "Two Compounds of Emetine Which May Be of Service in the Treatment of Entamoebiasis," published in the *Philippine Journal of Science*, Vol. X, No. 1, Section V, Tropical Medicine, January 1915, pages 75–79.

The second compound mentioned in the publication was emetine mercuric iodide which, however, has not found any extensive use.

Notes on Preparation.—The method outlined by DuMez consisted of precipitating an acidified aqueous solution of emetine hydrochloride with Dragendorff's reagent, collecting and washing the precipitate with water and drying it in the air at a temperature below 50° C. When so prepared, the compound has an ugly brick-red color. But if the product is made by precipitating an acidified aqueous solution of emetine hydrochloride with Dragendorff's reagent, then warming the mixture slightly until the maximum bright red color, consistent with no alteration of the emetine alkaloid, is attained, a more beautiful and more thoroughly combined product is obtained. The amount of heat required to effect the combination must be carefully controlled by experiment in order to prevent alteration of the emetine alkaloid, which is sensitive to heat under the conditions of manufacture of this compound. The difference in results of alkaloidal assays of this compound by gravimetric and volumetric methods is a measure of the heat control; the volumetric method giving results only for unaltered alkaloid, whereas the gravimetric method includes both altered and unaltered alkaloid. Therefore, a properly made compound should give results by both methods which fairly closely check each other.

The following table shows the results of assays, of a number of samples of emetine bismuth iodide from various sources, by both volumetric and gravimetric assay methods:

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[1] A paper on the toxicity of azulene by Dr. Loevenhart, Miss Whelan and Dr. Leonard, was read by the first-mentioned investigator at the Chicago (1919) meeting of the A. A. A. S. before the Pharmacological Society.

[2] "The Aims and Development of Phytochemical Research," *American Journal Pharmacy,* March 1917, p. 97.