SCIENTIFIC SECTION

PHYTOCHEMICAL NOTES.*

NO. 106. MONARDA PUNCTATA, L.†

BY A. A. HARWOOD.

Also for the purpose of supplementing earlier chemical work on this species, most of which had been done in connection with the volatile constituents thereof, a more careful study of the non-volatile constituents of the leaves was undertaken. This was particularly desirable for the sake of comparison with the non-volatile constituents of other species of Monarda in particular and of other labiates, in general.[1]

Extraction of Leaves.—47½ lbs. of powdered, air-dried leaves were extracted with 95 p. c. alcohol in the Lloyd extractor. After recovery of nearly all of the alcohol, the extract was steam-distilled. From the distillate, which contained some alcohol not previously recovered, the volatile oil was separated and the aqueous, or hydroalcoholic, portion cohobated. The non-volatile residue separated into an aqueous solution and an insoluble portion. Both were shaken separately and repeatedly with petroleum ether, the solvent being recovered in each case for further extraction. The petroleum-ether extracts were mixed. Hence there resulted the following products:

A. A volatile oil.
B. An aqueous (or hydroalcoholic) distillate.
C. A petroleum-ether extract containing fatty oil and other soluble constituents, such as chlorophyl, etc.
D. An aqueous solution of the water-soluble constituents of the original alcoholic extract after both volatile oil and fatty oil had been removed.
E. A powder consisting of the water-insoluble constituents of the original alcoholic extract after both volatile oil and fatty oil had been removed.
F. The dregs from the Lloyd extractor after the powdered leaves had been exhausted with 95 p. c. alcohol.

A. The Volatile Oil.—As previously indicated, the volatile oil (210 cc.) was obtained by steam distillation of the concentrated alcoholic extract. It was turned over to S. Chechik. Unfortunately the density was not determined. The phenol content, on two samples, as determined by shaking with 5 p. c. sodium hydroxide solution was found to be 89.0 p. c. and 89.1 p. c., respectively. The oil was reddish brown in color.

The aqueous distillate from the separated oil was cohobated as described later. The oil collected from the first cohobation amounted to 19 cc., was of a bright red color and had a specific gravity of 0.9501 at 22° C. The phenol content was found to be 97 p. c. and 98 p. c. on two samples. The second cohobation yielded 4 cc. of oil of a medium yellow color. The specific gravity at 22° C. was 0.9476. The phenol content was found to be 100 p. c. on the one sample.

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† Part of a thesis submitted for the degree of Doctor of Philosophy, University of Wisconsin. See also THIS JOURNAL, 19 (1930), 228; *Ibid.*, 19 (1930), 1171.

[1] The non-volatile constituents of *Mentha citrata* have been reported by S. Gordon (*Amer. J. Pharm.*, 100 (1928), 433 and 509), those of *M. piperita* by H. Braun (*Ibid.*, 102 (1930), 202).

All three oils are of an unusually high phenol content.

B. The Aqueous Distillate.—The aqueous distillate, from which the volatile oil had been removed, was cohobated until about $^1/_3$ its volume (approximately 10 liters) had been collected. The cohobate after removal of the oil was then redistilled, collecting again $^1/_3$ of its volume as before.

The final cohobate, after removal of another 4 cc. of volatile oil (see "A"), was fractionally distilled, the following fractions being collected (1) —65° C.; (2) 65–80° C.; (3) 80–85° C.; (4) 85–90° C.; (5) 90–95° C. Only a drop or so came over up to 65° C., hence only the last four fractions were refractionated with the following results.

		Amount.	Schiff's reagent.	Resorcinol sulphuric acid.	Gallic acid sol. + sulphuric acid.
(1)	—75° C.	0.5 cc.	+	+	+
(2)	75–76° C.	4.0 cc	+	+	+
(3)	76–78° C.	26.0 cc.	+	+	—
(4)	78–79° C.	72.0 cc.	—	—	—
(5)	79–80° C.	2.8 cc.	—	—	—
(6)	Residue (added to 90–95° C. above).				

The test with Schiff's reagent indicates the presence of an aldehyde in the first two and possibly the third fraction while the resorcinol-sulphuric acid test and the gallic acid-sulphuric acid test indicate the presence of formaldehyde. The second fraction gave a faint test for acetaldehyde when boiled with dilute sodium hydroxide solution while a red coloration was produced in the third fraction. When Fractions 3, 4 and 5 were heated with a small amount of glacial acetic acid with a few drops of sulphuric acid, the odor of ethyl acetate was quite perceptible—hence the presence of ethyl alcohol is indicated.

The test with sodium nitroprusside solution gave a negative test for the presence of acetone.

C. The Petroleum-Ether Extract.—This amounted to 1110 Gm. or 5.3 p. c. of the drug. It was saponified with an excess (about 25 p. c.) of a hydro-alcoholic solution of potassium hydroxide. The odor of ammonia becoming perceptible the reaction mixture was distilled, the vapors being collected in a 10-p. c. aqueous hydrochloric acid. Upon evaporation, only a small amount of residue remained, insufficient for the study of any possible amines.

The soap solution, from which the alcohol had been removed by distillation, was extracted repeatedly with ether. Evaporation of the solvent left 325 Gm. of unsaponifiable material. To the soap solution, a hot concentrated solution of lead acetate was added in slight excess under constant stirring. The lead precipitate was washed with hot water, dried and extracted with ether. The ethereal solution was filtered through a dry fluted filter paper. In this manner the ether-soluble compounds were separated from the ether-insoluble ones. In each case the lead compounds were decomposed with aqueous hydrogen chloride. The ethereal solution, after separation and evaporation of the solvent, yielded the so-called solid and liquid fatty acids, respectively. Thus the petroleum-ether extract was resolved into three portions, *viz.:*

I. Nonsaponifiable material (325 Gm.)
II. Solid fatty acids (176 Gm.)
III. Liquid fatty acids (119 Gm.).

I. Examination of Nonsaponifiable Material.—Inasmuch as the amount of non-saponifiable material seemed unusually large, a test for fatty material that might have escaped saponification was made, but with negative results. Acetylation gave 204.1 and 205.3 in two assays.

Although this material had been obtained from the original extract after steam distillation, it had a decided odor of thymol. In order to ascertain the approximate amount of phenol present, 3.4 cc. were shaken in a graduated cylinder with an excess of 5 p. c. sodium hydroxide solution. The volume was reduced to 2.5 cc., hence had suffered a diminution of 26.5 p. c.

In order to separate the phenols, the bulk of the unsaponifiable material was dissolved in heptane and the heptane solution extracted repeatedly with 5 p. c. aqueous sodium hydroxide. Before acidulating the separated aqueous solution it was shaken with heptane. The aqueous alkaline solutions, thus purified, upon acidulation with hydrochloric acid yielded the free phenols, which, upon standing, crystallized out.

A total of 104 Gm. of thymol, corresponding to 32.0 p. c. of the unsaponifiable, was obtained.

After the removal of the phenol as described, the heptane solution, upon standing, yielded a flocculent precipitate. Upon recrystallization from a large amount of heptane, the slightly yellow crystals obtained were recrystallized from a 50–50 solution of 95 p. c. alcohol and ethyl acetate. After a second recrystallization from the same solvent, the colorless crystals resulting gave the Liebermann color reaction for phytosterol. They melted at 136.5° C. The acetate, prepared in the usual manner, melted at 124.5° C. Phytosterols melt between 132–144° C., their acetates between 125–137° C.

The heptane filtrate from which the impure phytosterol had been separated was concentrated. Upon cooling the concentrate yielded a precipitate which was difficult to separate by filtration. Hence the heptane was removed completely and the residue dissolved in hot alcohol. Upon cooling, waxy material was obtained, readily soluble in hot, but sparingly soluble in cold alcohol. It melted at 68–70°. Some of this material was heated for an hour with acetic anhydride. The acetic anhydride was removed by evaporation and the residue crystallized several times from absolute alcohol. All of the acetylated product failed to go into solution in alcohol, so it was separated by filtration. The recrystallized acetylated product melted at 97–99° C. After repeated recrystallizations, the melting point rose to 104–105° C. The acetyl value as determined by saponification with $N/2$ alcoholic KOH was 107. The material from the determination of the acetyl value was freed from the soluble potassium salts by washing with water. After recrystallizing from alcohol, it melted at 127° C. and gave a positive Liebermann test for phytosterol. The melting point rose to 133° C. after repeated crystallization.

II. Examination of Solid Fatty Acids.—The methylation was carried out according to the method as outlined by G. D. Elsdon.[1] The 176 Gm. of solid fatty acids were refluxed with 400 cc. of anhydrous methyl alcohol[2] containing 2

[1] *Analyst*, 38 (1913), 8.

[2] The methanol was prepared by shaking stock methyl alcohol with burned lime for 24 hours and subsequent fractionation. Fraction 65° C. was reserved for the experiment.

p. c. HCl for 15 hours. Upon cooling, a semi-solid mass separated. The supernatent liquid was poured into 1600 cc. of water, from which solution the methyl esters were extracted with ether by repeated shaking. The combined ethereal extracts, in which was dissolved the semi-solid material referred to above, were washed with 1 p. c. sodium-carbonate solution in order to remove any hydrogen chloride that might be present. Upon evaporation of the solvent, 123 Gm.[1] of residue were obtained. The material thus obtained was a thick, dark green mass that was rather uninviting. An attempt to effect a separation by means of heptane seemed promising at first, but did not yield the expected results, hence was abandoned. Upon fractionation under diminished pressure (2.5 mm.) the liquid frothed at about 45° C. to such an extent that this method also had to be abandoned.

The methyl esters of the saturated fatty acids were, therefore, saponified with concentrated alcoholic potash. After removal of the alcohol, the resultant soap was broken up with 10 p. c. hydrochloric acid. The liberated fatty acids were then extracted with ether. However, it was found that the bulk of the material would not dissolve in ether but formed a layer at the junction of the acid solution. The ether layer was filtered and the ether removed. The ether-insoluble material was reserved for further investigation. Thirty grams of a semi-solid mass of the acids remained. An alcoholic solution of the acids was next prepared and this was treated while warm with a concentrated alcoholic solution of magnesium acetate in the ratio of $^1/_{30}$ of the weight of the acids. After the addition of the magnesium-acetate solution, the mixture was allowed to cool. The precipitated magnesium soaps were filtered off and dried. This procedure was repeated four times with a precipitate forming in each case. The fifth addition of magnesium acetate, however, caused no precipitation. The fatty acids from each precipitation were liberated by warming with dilute hydrochloric acid and the free acids shaken out with ether. After removing the ether, the acids were dried and the melting points determined. The melting points did not vary to any extent, all the samples melting from 54° to 57°. Inasmuch as there was only a small quantity, they were mixed. The combined acids were recrystallized several times from alcohol and several times from glacial acetic when their melting point was found to be 63° C. The neutralization value was determined on one sample and found to be 205.4. The acid from the neutralization value was liberated with dilute hydrochloric acid, decolorized with charcoal, and recrystallized from alcohol. The melting point was 63.0° C. Palmitic acid melts at 62.6° and has a neutralization value of 218.9. Stearic acid melts at 70–71.5° and has a neutralization value of 197.3. The melting point of the acid would indicate palmitic but the neutralization value is rather low.

The alcoholic solution, after all the magnesium soaps had been removed, was freed from solvent by evaporation. A viscid liquid remained which did not deposit crystals upon cooling. This material was dissolved in glacial acetic acid but nothing of a crystalline nature was obtained. It was probably some of the unsaturated fatty acids which were present due to the imperfect separation by the lead-salt ether method.

III. Examination of the Liquid Fatty Acids.—The liquid fatty acids were

[1] Owing to frothing in the methylation, some of the material was lost, hence the reduced yield.

separated from the solid fatty acids by the modified Gusserow-Varrentrapp lead salt-ether method.[1] The lead salts which were soluble in ether were liberated with 10 p. c. HCl and, after recovery of the ether, weighed 119 grams.

Oxidation of the Liquid Fatty Acids.—Thirty grams of the liquid fatty acids were oxidized according to Hazura's method[2] by treating an aqueous solution of the potassium soaps with a $1^1/_2$ p. c. solution of potassium permanganate. The precipitated manganese oxide was brought back into solution with SO_2 gas. The mixture was filtered and the precipitate treated with 500 cc. of ether at room temperature. After the solution had been filtered, the ether was recovered and a small amount of material obtained which, after one purification with alcohol, melted at 65–68°. However, the amount was too small to allow of purification. Nearly all of the precipitate was soluble in ether, indicating the absence of, or at least the presence of only a small amount of sativic acid.

The filtrate, from the first operation, had a volume of 4000 cc. This was evaporated down to a volume of about 350 cc. or approximately $^1/_{12}$ of the former volume. A flocculent precipitate came down which after several recrystallizations from alcohol melted at 202° C. but still retained some of the green color. The melting point of 202° C. would indicate linusic acid which is an oxidation product of linolenic acid. Linusic acid melts at 203–205° C.

Bromination of the Liquid Fatty Acids.—Ten grams of the fatty acids were dissolved in sufficient ether to make a 10-p. c. solution and cooled in an ice-salt bath; 2.5 cc. of bromine were then added slowly, with agitation, over a period of 35 minutes and another 2.5 cc. added over a period of 20 minutes. The ethereal solution was then allowed to stand for 2 hours. At the end of this period, the precipitate was filtered off and the ethereal solution washed several times with sodium thiosulphate solution to free it from excess bromine. This precipitate, after recrystallizing from benzene, melted at 180.5° C. The melting point of stearic acid hexabromide is 180–181° C. The bromine content determined on two samples was 59.2 p. c. and 60.4 p. c. Stearic acid hexabromide has a theoretical bromine content of 63.27 p. c. Tetrabromstearic acid has a melting point of 113–114° C. and a bromine content of 50.33 p. c.

The ethereal filtrate after washing with sodium-thiosulphate solution was distilled and the ether recovered. The residue was taken up with petroleum ether and the solution subjected to a low temperature but no crystals were obtained indicating the absence of linoleic acid.

The petroleum-ether filtrate was then subjected to distillation and the petroleum ether recovered. The bromine content of the residue was found to be 32.7 p. c. and 31.3 p. c., respectively, on two samples. Oleic acid dibromide has a theoretical bromine content of 36.17 p. c.

D. Examination of the Aqueous Extract.—Having been extracted with petroleum ether (see above, under "Extraction of Leaves") this extract was concentrated at a relatively low temperature and the concentrate shaken successively with ether, chloroform, etc. From the ethereal, chloroformic, etc., extracts the solvents were recovered. After the several extractions with these immiscible

[1] G. Lewkowitsch, "Chem. Tech. and Analysis of Oils, Fats and Waxes," 1 (London, 1921), 559.

[2] K. Hazura, *Monatsh.*, 9 (1888), 469.

solvents, the modified aqueous extract was treated as described below under chloroform extract.

In this manner the following products were obtained, *viz.:*

I. Material soluble in ether
II. Material soluble in chloroform
III. Material insoluble in chloroform.

I. Ether Extract.—After recovery of the ether, a syrupy liquid remained from which about 22 Gm. of crystals separated corresponding to 0.001 p. c. of the crude drug. Purified by sublimation, the white crystals melted at 140–141° C., the melting point of hydrothymoquinone. With thymoquinone they gave the thymoquinhydrone test. From the mother liquor, from which the hydrothymoquinone had been obtained, 9 Gm. of impure hydrothymoquinone were obtained by heating with water and chilling the solution. The aqueous filtrate was set aside for further investigation. It is less surprising that hydrothymoquinone should have remained behind in the steam distillation of the original extract than thymol, yet it is noteworthy that so much of this diatomic phenol should have escaped coming over with the volatile oil. True, when computed with reference to the crude drug, it amounts to but very little.

II. Chloroform Extract.—Upon concentration of the chloroformic extract and exposure of the concentrate to an ice-salt bath, long silky needles, green in color, no doubt because impure, separated which melted at 121° to 128° C. Recrystallized twice from alcohol the melting point had risen to 201° C. This substance was heated with dilute hydrochloric acid on a water-bath for 15 minutes and the cooled solution shaken with ether. The ethereal solution was added to an ethereal solution of thymoquinone on a crucible cover. Upon evaporation of the solvent, a blue-black residue suggestive of thymoquinhydrone was obtained. This indicates hydrothymoquinone as a product of hydrolysis of a possible glucoside. Further confirmation of the presence of a glucoside was obtained by neutralization of the aqueous acid solution which gave a positive test for sugar with Fehling's solution.

Anxious to obtain more of the glucoside thus indicated, the aqueous liquid which had been shaken repeatedly with chloroform was evaporated to dryness and mixed with purified sand and the material thus obtained subjected to extraction with chloroform for 12 to 15 hours in a continuous extractor. However, only a small amount of additional material was obtained from which even repeated recrystallizations failed to remove all of the green color. It melted at 201° C. which agrees closely with the melting point of several glucosides, especially with that of arbutin 200° C. (methyl arbutin melts at 175°). Upon hydrolysis of its aqueous solution a precipitate resulted. Purified from alcohol, it melted at 138–140° C. Whereas the quinhydrone test described above indicated a diatomic phenol but not necessarily hydrothymoquinone, this melting point distinctly indicates the latter.

III. Material Insoluble in Chloroform.—A small amount of the sand mixture was extracted with hot ethyl acetate. However, very little material seemed to be extracted, so the remainder of the sand mixture was reserved for further investigation.

SUMMARY.

(1) The volatile oil obtained by steam distillation of the alcoholic extract had a phenol content of 89 p. c.

(2) The presence of formaldehyde and acetaldehyde in the aqueous cohobate is indicated.

(3) The non-saponifiable material from the petroleum-ether extract was found to contain a considerable amount of thymol. If this amount of thymol is added to that in the volatile oil, the percentage is found to be 1.3 p. c., which is a surprisingly large yield.

(4) Linolenic, oleic and possibly palmitic acids were found to be present in the saponifiable portion of the petroleum ether extract.

(5) Hydrothymoquinone was identified and a glucoside of hydrothymoquinone was indicated in the water-soluble portion of the alcoholic extract.

THE USE OF ALUMINUM AND STANNOUS CHLORIDE IN THE GUTZEIT TEST FOR ARSENIC.*

BY LOUIS P. MAYRAND.

Analytical chemists have encountered two major difficulties in the detection of small amounts of arsenic by the Gutzeit and Marsh Methods. These obstacles are *first*, arsenic-testing zinc varies markedly in its purity, and *second*, it is very difficult to obtain this metal in a strictly arsenic-free state. Of these, the former is more conducive to poor results than the latter.

The zinc that is to be used for the evolution of hydrogen in these tests is not easily produced in an arsenic-free condition. The results of experiments indicate that some of the drillings from various sections of a supposedly arsenic-free zinc slab contain arsenic, whereas similar drillings from other sections of the same slab do not. The apparent variation in the arsenic contents throughout the zinc slab may be due as much to the difference in formation of active (*i. e.*, atomic) hydrogen caused by an uneven distribution of metallic impurities as to the irregularity in arsenic contents.

The purity of a metal plays a very important part in the rate of formation of active hydrogen. The rate of formation of active hydrogen is in turn an important factor in the reduction of a substance. In the Gutzeit and Marsh tests very small amounts of arsenic are usually dealt with. Unless a certain reducing action exists during the arsenic-testing reaction, the arsenic present may not be entirely reduced to arsine (AsH_3). That is to say, it appears that it is difficult to displace the last traces of arsenic introduced into the reaction.

The metals which occur below hydrogen in the potential series are capable of causing an evolution of hydrogen when introduced under suitable conditions into a dilute solution of an ionizable acid. The velocity of the reaction varies to a marked extent with the purity of the metal. Pure zinc, for instance, is almost unattacked by pure dilute sulphuric acid, but if the zinc be touched with a copper rod below the surface of the acid, solution of the zinc at once commences with

* Northwestern Branch, A. PH. A., May 1, 1930.