

where heptyl hydride is supposed to have been added to halogen which has assumed trivalence to make possible this addition. Naturally, all we know about the valence of the halogens causes us to believe that such a compound, if formed, will be very unstable indeed. As, *e. g.*, the HCl addition product of cineol, a completely saturated compound itself, is broken up into its components by water, so the heptyl hydride "compound" of HCl may be even less stable. This explanation is given here for what it is worth merely to guard against any dogmatic statements about purely physical solution as the only way to account for the phenomena here recorded.

II. A year or two ago, an associate in plant physiology called me up over the 'phone wanting to know whether carbon dioxide is soluble in petroleum. It was one of those questions that will cause persons with some sense of humor left to smile rather audibly. No doubt, many if not most of our examiners of candidates for the doctor's degree find questions of this sort perfectly proper, but these seldom have the saving grace of the class referred to above. The sense of humor was doubly keen in this case because the writer was in a position to give an answer to the question. This possibility demands an explanation.

In connection with work being done on pinene, the question of the solubility of carbon dioxide in this hydrocarbon came up and the only answer the writer could give was: "Try it out." The results of the experimental "try out" are recorded in a separate report.¹ Inasmuch as pinene is an unsaturated hydrocarbon, the possibility of chemical addition was not excluded from a purely theoretical point of view, though the results showed that any chemical compound, if formed at all, was, for the most part, exceedingly labile. It was of some interest, therefore, to ascertain something about the solubility of carbon dioxide in heptane. Benefiting by the previous experience with pinene, the heptane was directly supported in the graduated tube of a Lunge nitrometer by lime water instead of water. In a comparatively short time the CO₂ had passed through the heptane as was indicated by the formation of calcium carbonate in the lime water. Unfortunately the notebook in which these experiments have been recorded is lost, hence no exact data as to time can be given at present.

PHYTOCHEMICAL NOTES.*

90. The Seeds of *Bixa Orellana*.

BY S. S. AIYAR.

The seeds of *Bixa orellana* L., which yield the Annatto, have been investigated repeatedly because of their pigment which is used extensively in the dairying industries. Other constituents of the seed have received little or no attention.

The immediate impulse for this preliminary investigation along more general lines was twofold: First, the close relation between this species and that yielding chaulmoogra oil[†] and the medical importance which the latter has acquired recently, suggested an examination of the fatty oil for chaulmoogric acid; second, a visit to Mr. Marshall's factory in this city and the sight of a large quantity of

¹ W. F. Sudro, "Absorption of Gases by Pinene."

• From the laboratory of Edward Kremers.

[†] † For references see Bibliography at end of article.

seed that goes to waste after the pigment has been washed off well might impelled the visitor to use at least a small part for scientific purposes.

MOISTURE.

The red seeds, when ground, were subjected to distillation with xylene in order to determine the moisture content. The yield was 3.0 percent and 3.1 percent, respectively. The xylene left in the distilling flask was filtered while hot. The filtrate was dark orange in color and upon cooling precipitated the orange-colored dye which it had dissolved while hot.

The waste seeds which in the factory had been deprived of their pigment coating yielded but 2.3 percent and 2.1 percent, respectively, of water. The lower moisture content is readily understood when one recalls that these had been dried artificially after having gone through the manufacturing process.

ASH.

Ash determinations of both the original and processed seeds were made. The results are herewith tabulated:

	Original seeds, percent.		Processed seeds, percent.	
	I.	II.	I.	II.
Insoluble ash.....	2.43	2.27	3.35	3.52
Soluble ash.....	2.39	2.46	2.47	2.44
Total ash.....	4.82	4.73	5.83	5.97

The larger soluble ash content, hence also a larger total ash content, is readily understood when one recalls the method of processing employed.

ETHER EXTRACTIVE.

The close botanical relation of this plant to that yielding the chaulmoogra oil made it particularly desirable to ascertain something about its fatty oil content as well as about the nature of the fatty oil. For this purpose 100 Gm. of finely powdered processed seed were extracted in a Soxhlet with ether for eight hours. The processed seed was taken for this experiment in order to avoid the complications that would arise from the red pigment. The ether solution was yellow, not red. Upon evaporation of the ether there remained 2.55 percent of residue which was liquid in part, and in part consisted of small crystals. A second extraction with ether added but little, about 0.2 percent, to this residue.

HEPTANE EXTRACTIVE.

Inasmuch as it was decided to subject larger amounts of seed to extraction with petroleum ether, it was deemed desirable first to extract on an analytical scale with heptane. Hence 125 Gm. of finely ground seed were extracted in a Soxhlet with heptane for about 8 hours. Again the heptane solution was yellow, not red, showing that all of the red pigment had been removed in the processing. Upon recovery of the heptane, a residue amounting to 2.8 percent remained. It also was oily in part, and in part consisted of small acicular crystals.

ALCOHOLIC EXTRACTIVE.

The powdered seeds, after having been exhausted with ether, were transferred to a flask and extracted with boiling alcohol for three hours. The yellow alcoholic solution was filtered while hot and the seeds extracted a second, third and even a fourth time by boiling with alcohol. The residue from the alcoholic

solution was ropy in consistence and amounted to 9 percent of the processed seeds. The seeds previously extracted with heptane gave 9.4 percent of alcoholic extract.

FATTY OIL.

In order to obtain a larger amount of fatty oil $25\frac{3}{4}$ pounds of crushed seeds, from which the pigment had been removed in the factory, were extracted in a large extraction apparatus with "fractol" for about ten days. Even then the extraction was imperfect as is shown by the yield, which amounted to but 1.7 percent. The oil was a thick greenish brown liquid.

Inasmuch as the oil was too dark in color to admit of the determination of certain constants, a part of it was distilled under a pressure of 8 to 12 mm. under which conditions 60 percent passed over between 190° and 210° . Freshly distilled the oil was pale yellow in color and became semi-solid at ordinary temperatures and on keeping it turned slightly brown. When liquefied it was distinctly brown in color. The forty percent residue was rubber-like, resembling the residue from castor oil in the production of heptenal by destructive distillation.

Such constants as were determined are herewith tabulated together with those recorded for chaulmoogra oil:

Constants.	<i>Bixa orellana</i> crude oil.	<i>Bixa orellana</i> dist. oil.	Chaulmoogra oil. ²
S. V.	227.8	166	208.0
I. V.	124.9	116.6	104.4
A. V.	17.3	117.9	9.5
α_D^{15}	0	+51.3°
d_{25}	0.928	0.914	0.952
n_D	1.4745 at 28°
M. p.	Liquid	290°	22-23°

The great diminution in the saponification value and the corresponding increase in the acid value of the rectified oil show that decomposition of the glycerides must have taken place even under the greatly reduced pressure under which the distillation was conducted. Under these circumstances the practical absence of optical rotation of the rectified oil may signify nothing as compared with the high angle of rotation of chaulmoogra oil. More significant is the greater density of the latter as compared with that of the crude *Bixa orellana* oil.

In order to ascertain whether the *Bixa* oil contained chaulmoogric acid, the method adopted by Power and his co-workers³ was tried out on the distilled oil, but the results proved negative. Inasmuch, however, as but 30 Gm. of material were available, this experiment is to be repeated on a larger scale as soon as convenient.

In order to separate any oleic acid that might be present from saturated fatty acids the method of Gusserow and Varrentrapp,⁴ as described by Leukowitsch,⁵ was employed. About 16 Gm. of mixed fatty acids were available. The lead salts from 7.3 Gm. separated by ether yielded 1.2 Gm. of solid and 4.7 Gm. of liquid fatty acids. In another experiment 8.7 Gm. gave 2.1 Gm. of solid and 5.1 Gm. of liquid fatty acids. That the separation was not absolutely quantitative was shown by the residue of unoxidizable solid fatty acids when the liquid acids were oxidized with permanganate.

The solid fatty acids were soluble in hot 75 percent alcohol from which they were recrystallized. The acid thus purified melted at 58° whereas palmitic acid

melts at 62.6° and stearic acid at 69.3°. The saponification value was found to be 205, whereas that of palmitic acid is 219. Inasmuch as a mixture of 80 percent palmitic acid and 20 percent stearic acid melts at 56.5° it is probable that the solid acids obtained consist principally of palmitic acid with some stearic acid.

The liquid acid or acids were oxidized with permanganate according to the directions given by Leukowitsch.⁶ The oxidation product is apparently dihydroxy stearic acid which melted at 133° and which in all probability resulted from oleic acid present. Inasmuch as only 5 Gm. of liquid acid were available for this experiment, it is impossible to state whether small amounts of other acids are present or not.

A qualitative test for phytosterol indicated the presence of this substance in the non-saponifiable oil. However, the amount was too small to admit of any more definite statements.

SUGARS AND GLUCOSIDE.

The ropy material left upon evaporation of the alcoholic tincture was freed from fatty oil (as stated the petroleum ether extraction had been imperfect) by washing with ether. Some coloring matter and possibly other impurities were removed by washing with cold alcohol. The material thus purified was dissolved in water, the solution treated with lead acetate and the excess of lead removed from the filtrate with H₂S. The filtrate from the lead sulphide yielded a non-crystallizable residue. Dissolved in water this reduced Fehling's solution somewhat and gave a small amount of osazone melting at 200°. Previously hydrolyzed with 5 percent hydrogen chloride, the modified solution reduced Fehling's solution readily and gave an abundant osazone precipitate, thus indicating the presence of a glucoside as well as of a reducible sugar.

About 44 Gm. of material were then hydrolyzed. The distillate gave no test for hydrogen cyanide, thus indicating that the glucoside is non-cyanogenetic. The distillate had the odor of the aqueous distillate of the seeds, but whatever the substance was it defied identification in such small amounts.

While hydrolyzing the glucoside a brown, almost black powder was precipitated. After hydrolysis this was separated from the deep brown solution which could not be decolorized with animal charcoal. The precipitate was soluble in hot alcohol and upon evaporation of the solvent was obtained as dark brown scales.

Two methoxy determinations according to Zeisel⁷ gave 1.49 and 1.93 percent, respectively. According to Zwick⁸ and Etti⁹ bixin has the formula C₂₈H₃₆O₅ and contains one methoxy group, namely, 6.9 percent. Van Hasselt, on the other hand, assigns to bixin the formula C₂₉H₃₆O₅ which brings the methoxy percentage slightly lower, *viz.*, 6.7 percent. However, the methoxy content of the precipitate is much lower. Such relation as may exist between bixin, the red coloring matter of the seed, and this product of hydrolysis of the glucoside could not be ascertained from the small amount of material available.

Owing to this coloring matter, it has been impossible thus far to obtain the sugar produced by the hydrolysis in a pure condition.

STEAM DISTILLATION OF THE SEEDS.

Twenty-six and a half pounds of crushed seed were subjected to steam distillation. The aqueous distillate of 15 gallons had a distinct odor, but no oil separated.

Hence it was cohobated, five liters being collected and, upon a second cohobation, 100 cc. This last distillate was opaque and a thin film of oil separated, too small, however, for examination. As already pointed out this distillate had an odor similar to that of the distillate obtained upon hydrolysis of the glucoside.

It is hoped that this preliminary investigation of the seeds of *Bixa orellana* may be repeated with much larger quantities of material.

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1. According to Engler and Prantl the *Flacourtiaceae*, to which *Taraktogenos Kurzii*, from which chaulmoogra oil is obtained, and the *Bixineae*, to which *Bixa orellana* belongs, are closely related families. According to Bentham and Hooker the chaulmoogra yielding plant is classified under the *Bixineae*.
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3. *Ibid.*
4. *Liebig's Annalen*, 27, 153, 1828; also 35, 197, 1836.
5. "Technology of Fats and Waxes," Vol. I, p. 442.
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7. *Monatsh. f. Chem.*, 6, 989, 1885; 7, 466, 1896; also Zeisel & Fanto, *Zeitsch. anal. Chem.*, 42, 549, 1903; Stritar, *Ibid.*, 42, 579, 1903.
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ELECTROMETRIC ASSAY METHODS FOR CRUDE DRUGS.*

BY WILLIAM J. MCGILL AND PAUL ELLIS FAULKNER.

One of the most fruitful developments of the electrolytic dissociation theory announced by Arrhenius in 1887 has been the quantitative conception of acidity and alkalinity. Previously, solutions were termed acid, alkaline or neutral, and no attempt was made to interpret different degrees of acidity or alkalinity, although Pasteur had early recognized the effects of these on the growth of microorganisms.

The term "hydrogen-ion concentration" has been used to designate degree of acidity or alkalinity. Since the product of the hydrogen and hydroxyl ions in any aqueous solution must always equal 1.10^{-14} a definite concentration of H-ion is always present, whether the solution be alkaline, acid or neutral, and it is therefore proper to speak of the H-ion concentration of a neutral or alkaline solution as well as of an acid solution. A further development in nomenclature has been the use of the term " p_H value" in place of the more cumbersome "H-ion concentration." The p_H value corresponds to $\log 1/(H^+)$ and the p_H values for different H-ion concentrations may be calculated from this equation.

The working out of methods for the determination of H-ion concentration has been largely done by Sørensen and his assistants at Copenhagen. Michaelis, in 1914, published his monograph, "Die Wasserstoffionenkonzentration," which summarized the entire subject up to that date. Since that time countless journal articles have appeared dealing with new methods of, and new applications for, the determination of H-ion.

So far, the development of this field has been due in great measure to the desire of workers in the biological sciences for a better knowledge of the effects of

* Scientific Section, A. Ph. A., Cleveland meeting, 1922.