corresponding dichromate solution. The use of ceric sulphate as an oxidizing volumetric reagent has been extensively studied by Smith (4).

EXPERIMENTAL.

A sample of Mass of Ferrous Carbonate was prepared according to the directions in the U. S. P. X. The theoretical yield of ferrous carbonate in the mass is 41.5 per cent.

This mass was assayed using potassium dichromate tenth-normal solution with diphenylamine T.S. as the indicator. Using this method, the mass assayed above the theoretical amount of $FeCO_3$, namely, 43.2 per cent. This higher value is in conformity with the findings of Hartley and Linnell (5) who suggested that the oxidations occur in the following order: ferrous iron-carbohydrate indicator. Using tenth-normal ceric sulphate as the volumetric reagent, the mass showed the following percentages of ferrous carbonate: 40.6, 40.4, 40.4, 40.5, 40.5, 40.3, 40.6 and 40.6. When the pure ferrous sulphate is titrated in the absence of honey and sugar, the results with the two volumetric reagents are identical.

The results using ceric sulphate are closer to the theoretical amount of ferrous carbonate and the end-point is much sharper. Ortho phenanthroline, an indicator recommended by Smith (4) in the titration of ferrous salts with ceric sulphate, gave satisfactory results and a strikingly sharp end-point.

The following procedure is recommended.

Dissolve about 1 Gm. of Mass of Ferrous Carbonate, accurately weighed, in 15 cc. of diluted sulphuric acid, add 10 cc. of diluted phosphoric acid and 100 cc. of distilled water. Immediately titrate with tenth-normal ceric sulphate, using 0.5 cc. of diphenylamine T.S. as the indicator. Each cc. of tenth-normal ceric sulphate is equivalent to 0.01159 Gm. of $FeCO_3$.

CONCLUSION.

Ceric sulphate solution has been advantageously employed in the assay of Mass of Ferrous Carbonate.

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STUDIES ON CUDBEAR.*

BY E. H. WIRTH, L. E. MARTIN AND P. G. SODERDAHL.[1]

Cudbear as a coloring agent for pharmaceutical preparations came into use about 1874 (1) and in spite of complaint involving principally its lack of uniformity, it has enjoyed considerable popularity. Although the tincture was previously official, cudbear as such, did not appear until the N. F. IV, where it was defined as

* Scientific Section, A. PH. A., Portland meeting, 1935.

[1] University of Illinois, College of Pharmacy, June 1935.

"A purplish red powder prepared from species of *Rocella, Lecanora* and other lichens." The monograph in the N. F. IV included tests for brazilwood, logwood and coal-tar colors, and allowed 35% ash. No marked change was made in the N. F. V except that the maximum ash limit was placed at 12%.

Various attempts have been made to replace cudbear with one or another of the synthetic organic dyes, the most favorable one being amaranth suggested by Morrison (2) who offers as his objections to cudbear, the hinderance in filtration, color changes in acid and alkaline solution, fading and lack of uniformity. (3) The bulletins of the N. F. VI, however, show considerable controversy (4) over the replacement of cudbear with amaranth in N. F. formulas, the objections to change being that amaranth does not impart the same color as cudbear, it therefore being unwise to change the color of established preparations; that it is satisfactory; that no lack of uniformity has been observed; and that some states have regulations restricting the use of coal-tar dyes. The controversy finally resulted in the committee voting for the retention of cudbear. At the request of Chairman Gathercoal the studies published herewith were made in connection with the revision of the cudbear monograph.

Earlier objections to the use of cudbear were based particularly upon the lack of uniformity of tinctures made from various lots. Attempts by Gardner and Raubenheimer (5), Arny (6) and Beringer (7) to prepare extracts of cudbear for use in coloring pharmaceutical preparations did not meet with considerable success. In the cudbear of that time it had been noted that sodium chloride (8) seemed to be present in many samples in considerable quantity. Craig (9) among others reported the lack in uniformity in color, concentration and tint. The N. F. IV seemed to solve the variation in the color of preparations colored with tincture of cudbear by substituting cudbear itself in the formulas. It, however, still allowed 35% of ash which did not remedy the sodium chloride situation. In the N. F. V the ash limit was reduced to 12% which then resulted in the disappearance of sodium chloride as a diluent.

Cudbear belongs to that interesting group of dye substances, orchil, cudbear and litmus, which are commercially produced from various lichens, principally *Roccella* and *Lecanora*. Beringer (7) (1912) mentions *Lecanora tartarea* of Northern Europe as the principal source. Most of the present-day cudbear, however, is produced in the Canary Islands, Madagascar and on the African coast.

Very little definite information relative to the manufacture of cudbear is available, the manufacturers apparently guarding their secrets rather closely. In general, however, the lichens contain colorless glucosides (?), acids and ester-like compounds of orcin which upon oxidation in the presence of ammonia split first into orcin, a colorless compound and are then converted into orcein and other colored substances. The variety of colored substances produced is rather considerable and is discussed at length by Beringer (7). Altering the source of the lichens or modifying the process and the alkali used produces different end products which result in the different commercial substances.

Cudbear itself is usually produced by digesting the lichens with about three times their weight of solution of ammonia at 60° for from three days to a week, air being admitted as a considerable requisite. The mixture assumes first a blue and subsequently a red color when the product is dried and ground.

The problem then consisted (1) in studying the uniformity of cudbear; (2) in studying its quality and purity and finally (3) in establishing, if possible, standards to insure these points. To this end 16 samples were obtained from various sources representing in a fair degree the average cudbear on the market. The samples were as follows:

Sample 1. U. of I. Pharmacognosy Museum. Sample about 40 years old.
Sample 2. Local druggist's stock. Age and source unknown.
Sample 3. Mass. College of Pharm. Age and Source unknown.
Sample 4. U. of I. Coll. of Pharm. Dept. of Pharm. stock. Purchased 1933 from a New York wholesale house.
Sample 5. Local druggist's stock. Purchased 1929 from Chicago wholesale house No. 1.
Sample 6. Purchased from Chicago wholesale house No. 2, 1932.
Sample 7. Purchased from Chicago wholesale house No. 3. Age unknown.
Sample 8. Local druggist's stock. Age and source unknown, apparently old.
Sample 9. State Ed. and Research Hosp. Pharmacy. Purchased 1931 from a New York wholesale house.
Sample 10. State Ed. and Research Hosp. Pharmacy. Purchased 1933 from a Chicago wholesale house.
Sample 11. Chicago wholesale house No. 4. Received by them from foreign dealer "A." 1934.
Sample 12. Chicago wholesale house No. 4. Received by them from foreign dealer "B." 1934.
Sample 13. Chicago wholesale house No. 4. Received by them from foreign dealer "C." 1934.
Sample 14. Chicago wholesale house No. 4. Received by them from foreign dealer "D." 1932.
Sample 15. Chicago wholesale house No. 4. Received by them from foreign dealer "E." 1929.
Sample 16. Southern Illinois wholesale house. 1934.

COLORIMETRIC VALUE.

Since cudbear is employed solely as a coloring agent, a study of the colorimetric value of the samples at our command seemed the best way to determine their quality. The method suggested by Scoville (10) employing standardized solutions of inorganic chemicals as devised by Arny and his co-workers (11) appeared well adapted to this purpose. The method is as follows:

"Accurately weigh one gram of Cudbear previously dried over sulphuric acid; macerate it for 18 hours in 100 cc. of a mixture of 3 volumes of alcohol and 1 volume of water, cooled to room temperature before measuring. Shake frequently and allow the drug to settle. To 5 cc. of the clear liquid, accurately measured, add 15 cc. of alcohol, then gradually add distilled water to make 1000 cc. and mix. Compare the color of this freshly prepared solution in Nessler tubes or in a colorimeter with the color of a standard color solution prepared as follows:

Decinormal Cobalt Chloride	0.75 cc.
Two-hundredth-normal Potassium Dichromate	0.30 cc.
Ammonium Carbonate T.S.	3.00 cc.
Distilled water, a sufficient quantity to make	10.00 cc.

The color of the cudbear solution should not be less than that of the standard solution prepared above."

In carrying out the method the standard solution was set in the colorimeter (B. & L. Biological No. 2400) at a depth of 20 mm. The cudbear solutions were adjusted to equalize the color field, and depth readings taken. Several readings (often by several observers) were averaged and recorded. Two macerations were made from each sample and are designated as "A" and "B." Factors were then calculated for each sample indicating its color intensity as compared with 1.00 =

the standard. (Thus, a sample in which the depth reading is less than that of the standard will consequently have a factor larger than 1.00, it being stronger than the standard.) Results are given in Table I.

TABLE I.

Sample No.	Depth in Mm. of Samples. "A."	"B."	Average Depth in Mm.	Factor.
1	18.5	18.1	18.3	20/18.3 = 1.09
2	18.3	18.5	18.4	20/18.4 = 1.08
3	16.4	15.3	15.85	20/15.85 = 1.26
4	17.3	17.0	17.15	20/17.15 = 1.17
5	20.7	20.8	20.75	20/20.75 = 0.97
6	19.5	19.3	19.4	20/19.4 = 1.03
7	16.0	15.7	15.85	20/15.85 = 1.26
8	22.5	22.7	22.6	20/22.6 = 0.88
9	23.8	24.5	24.15	20/24.15 = 0.83
10	20.5	21.3	20.9	20/20.9 = 0.96
11	16.5	15.7	16.1	20/16.1 = 1.24
12	17.0	16.5	16.75	20/16.75 = 1.19
13	15.6	15.0	15.3	20/15.3 = 1.30
14	12.3	12.5	12.4	20/12.4 = 1.61
15	17.3	17.1	17.2	20/17.2 = 1.16
16	17.3	17.0	17.15	20/17.15 = 1.17

COMMENTS ON COLORIMETRIC RESULTS.

(1) Color tints between samples and standard check fairly close, at least close enough to make a semi-quantitative estimation of color. Slight variations in tint between samples may be due to variation in manufacture, or in rare cases to the presence of added coloring substances. In the sixteen samples examined only one such variation was observed, and this was not great enough to materially affect colorimetric observation.

(2) The standard solution should be freshly prepared and should be used at once due to the volatilization of ammonia. The three component solutions used in its preparation, however, are stable, and may be kept as stock solutions. The Ammonia T.S. should, of course, be kept tightly stoppered and the usual precautions involving the effect of ammonia on glass observed. The cobalt chloride and the potassium dichromate solutions should be standardized by the usual chemical means.

(3) Although the strongest sample (No. 14) was twice that of the weakest (No. 9) in colorimetric strength, both were extreme as compared with the entire group. The surprising thing is that for a substance reputed to be so carelessly prepared, the results run astoundingly close. This suggested to us that the manufacturers were employing some means of keeping the color value of cudbear more or less constant. No reference to such a fact appears in the literature or any textbook at our command. Our color findings, however, led us to make a microscopical examination of the cudbear samples with further surprising findings. These results appear elsewhere in this paper. Rather extended subsequent correspondence with foreign and domestic dealers in cudbear, however, substantiated our supposition. It seems to have been a known fact to them that for some time diluents have been used commercially.

(4) It will be observed that 12 samples (75%) ran above standard and 4 (25%) ran below standard. Of those above standard 8, or $^2/_3$ of them ran over 10% above standard, while of those below standard 2 or $^1/_2$ of them ran more than 10% below standard. The standard, therefore, as suggested by Scoville seems to be a fair one. Of the sixteen representative samples only two ran more than 10% below the standard.

SOLUBILITY.

As a possibility for determining the quality of cudbear a solubility method suggested itself. Approximately 70% alcohol (the tincture menstruum) appeared to be the most likely solvent for such a determination. Solubility tests were run in

conjunction with the colorimetric determinations as follows: After a small portion of the supernatant liquid had been withdrawn for the colorimetric tests from the 18-hour macerations, previously described, the balance of each was filtered through a tared filtering crucible, the residue being washed with 70% alcohol until the washings were colorless. The residue was then dried at 100° and weighed, the weight obtained representing that portion of the 1-Gm. sample of cudbear insoluble in 70% alcohol. The results are given in Table II.

TABLE II.—SOLUBILITY.

Sample No.	Percentage of Residue Insoluble in 70% Alcohol. "A."	"B."	Average of A & B.	Color Factor (Table I).
1	63.02	62.48	62.75	1.09
2	69.44	69.65	69.54	1.08
3	65.60	65.17	65.38	1.26
4	72.57	73.22	72.89	1.17
5	69.25	68.91	69.08	0.96
6	69.08	69.42	69.25	1.03
7	67.02	66.10	66.56	1.26
8	66.21	66.04	66.13	0.88
9	83.62	83.18	83.40	0.83
10	83.57	82.79	83.18	0.96
11	73.55	73.52	73.54	1.24
12	73.05	74.00	73.53	1.19
13	65.32	65.15	65.24	1.30
14	68.46	68.97	68.72	1.61
15	73.25	73.32	73.29	1.16
16	65.74	66.12	65.93	1.17

COMMENTS ON TABLE II.

While it is true that some of the samples which run high in color value show a low percentage of material insoluble in 70% alcohol (*e. g.*, No. 3 and No. 13) and that some of the samples which run low in color value show a high percentage of material insoluble in 70% alcohol (*e. g.*, No. 9 and No. 10) it can readily be observed that there is no consistent agreement between colorimetric and solubility results. Solubility results are therefore no criterion of the tinctorial value of cudbear. A comparison of the results, however, does seem to indicate that there may be substances present in some of the samples which do not impart a color but still go into solution in 70% alcohol. It is also interesting to note that between 62% and 83% of cudbear is insoluble in 70% alcohol.

PURITY.

As was previously pointed out the earliest foreign matter reported (8) present in cudbear was sodium chloride. To eliminate this the N. F. V reduced the ash limit from 35% to 12%. In order that our cudbear studies be as complete as possible ash determinations were run on the samples. These appear in Table III.

TABLE III.—TOTAL ASH.

Sample No.	Percentage Total Ash. A.	B.	Av.	Color Factor (Table I).
1	13.74	13.92	13.83	1.09
2	7.3	7.55	7.48	1.08
3	4.96	4.97	4.96	1.26
4	4.29	4.55	4.42	1.17
5	6.14	6.21	6.18	0.96

6	9.58	9.76	9.67	1.03
7	6.55	6.45	6.50	1.26
8	23.87	24.22	24.05	0.88
9	10.43	10.31	10.37	0.83
10	8.76	9.12	8.94	0.96
11	4.72	4.86	4.79	1.24
12	4.69	4.51	4.60	1.19
13	5.11	5.40	5.26	1.30
14	16.05	16.08	16.07	1.61
15	13.47	...	13.47	1.16
16	4.09	...	4.09	1.17

COMMENTS ON TABLE III.

It will be observed that 75% of the samples run well under the N. F. ash limit of 12%. Only one sample (No. 8) runs excessively above this limit. Samples 1 and 8 were definitely known to be of considerable age and therefore suspecting their high ash content to be due to the presence of salt, they were extracted with water and gave the customary microchemical tests for NaCl. Samples 14 and 15, however, gave only traces of chlorides.

Color factors have again been carried over from Table I. As is to be expected there is no correlation between ash and color value: *e. g.*, No. 14, the sample of highest color value runs considerably over the ash limit while No. 16, the sample lowest in ash is only average in color value. On the other hand, however, samples No. 8 and No. 9 do show high ash and low color value while No. 3, No. 11 and No. 13 show low ash and high color value.

There is always a question as to the selection of an ash limit. The present limit of 12% is probably a satisfactory one. At least it is not severe since half of the samples examined ran under 6.5%.

MICROSCOPICAL.

As was previously stated our colorimetric and other analytical results indicated that a microscopical examination of the samples might throw some light on the question of their purity. Such an examination was made, both the original samples and the residues from the (70%) alcoholic extractions being observed. As was to be expected with a substance of the nature of cudbear, the mounts showed considerable quantities of extraneous matter. The most surprising foreign substance found was potato starch, present in variable amounts in about half of the samples and indicating most conclusively that starch is being added as a diluent, presumably to regulate the color value. Since the starch was that of the potato (except No. 13, which was wheat) this adulteration no doubt takes place abroad.

Several other tissue elements, not of lichen origin, were found in many of the samples, the most common being woody tissues and hairs (presumably from leaves). These apparently originate from plant parts either gathered with the lichens through carelessness or added intentionally to the lichen mass during or before treatment.

Hyphæ from the subhymenial layer of the lichen are abundant in the mounts occurring occasionally as tissue fragments but more often torn apart and more or less broken. The hyphæ vary considerably in length and are from 0.002 to 0.006 mm. in diameter. In rare cases they show branching. Fragments of the undifferentiated pseudo-parenchymatous portions of the lichens may also be seen especially after clearing with chloral.

Most of the woody tissue appears to be of a dicotyl source, although coniferous wood was found in some samples. We were unable to identify the botanical source

of either the woody tissue or the hairs. It is highly doubtful whether the woody tissue found was from brazilwood, logwood or other colored woods. It seems rather to be a local adulterant entering through carelessness, being often accompanied by fragments of various bark tissues notably stone cells.

The microscopy of cudbear presents some difficulties. The phloroglucin-hydrochloric acid reaction for lignin cannot be used since the lignified tissue present is already colored pink or reddish by the natural coloring principle of the cudbear. Attempts to remove this coloring principle were more or less unsuccessful. Alcohol will not extract it from the tissue elements. We were, however, able to reduce the dye to its colorless base with sodium hydrosulphite. This reagent, however, also removes the lignin and is therefore of no use in preparing the sample for lignin testing. Identification of woody tissue could be and was based upon the presence of its characteristic elements, tracheæ, tracheids, wood fibres and fragments of medullary rays. Mounts allowed to remain 12–24 hours in chloral became sufficiently clear to facilitate the study of these elements. For the sake of brevity the microscopical results are tabulated in Table IV.

TABLE IV.—MICROSCOPIC.

Sample No.	Potato Starch.	Woody Tissue.	Hairs.	Remarks.	Color Factor.
1	0	+	0		1.09
2	0	++	0		1.08
3	+	+	0	Both pine and dicot. wood	1.26
4	+++	0	0		1.17
5	0	++	+	Hairs long, unicellular	0.96
6	0	++	0		1.03
7	0	+	0	Wood mostly pine	1.26
8	0	+++	0	Dicot. and pine wood	0.88
9	+++	+	+++	Stone cells in groups, hairs unicellular, often in clusters	0.83
10	++	+++	+++	Hairs long, unicellular	0.96
11	0	+++	0		1.24
12	+++	+	0		1.19
13	++ (wheat)	0	+	Endosperm cells of wheat, cross cells of wheat bran	1.30
14	0	+	+	Few curved unicellular hairs, occasional stone cells	1.61
15	+	0	+++	Wavy unicellular hairs, stone cells in groups	1.16
16	+++	0	+		1.17

Explanation of figures.

0 = absent
+ = present
++ = considerable
+++ = abundant.

DISCUSSION OF TABLE IV.

The microscopical studies show that:

(1) Where considerable potato starch is present (4, 12 and 16) the color value is fairly constant (about 1.17), except in No. 9 which appears to be grossly adulterated and is low in color value. Samples 4, 12 and 16 appear to be reduced to a definite color value.

(2) No. 13 is diluted with ground whole wheat.

(3) Where an abundance and a variety of foreign matter occur (9 and 10) the color value is usually low. Samples 9 and 10 also show a high residue insoluble in 70% alcohol (Table II) and No. 10 is excessively high in arsenic. These particular samples may therefore be exceptions. Samples 13 and 14 on the other hand show some foreign matter and are high in color value.

(4) Woody and leaf tissue show no correlation with color value and appear to be present due to carelessness in collecting and treating the lichens.

(5) All samples contain some foreign matter or other, and it may reasonably be said that no such a thing as a "pure sample" of cudbear can be found on the market. By a pure sample is meant one containing only lichen tissue and the oxidized color base.

(6) The addition of starch as a diluent, which seems to be extensively practiced, brings up the question whether such a diluent should be permissible. Both the Pharmacopœia and the National Formulary have in the past specified and permitted inert diluents for certain drugs. Starch is, of course, an inert diluent. If a color standard is to be established there will be a natural tendency to meet it but not to exceed it. If the object of the color standard be that of insuring a uniform drug a specified diluent should be permitted. If, on the other hand, it be the object of the standard to insure the quality of the drug, no such diluent should be permitted and an effort should be made to prohibit within reasonable limit all foreign matter. The standard should then be placed high enough to insure care in manufacture and discourage dilution.

WOODY ADMIXTURE.

Certain woods containing coloring principles seem to be suspected adulterants of cudbear. In the past several editions, the N. F. has included tests for brazilwood and logwood. It would seem, however, that adulteration with these and other colored woods whose habitats are far removed from those regions where cudbear is produced, would be of rare occurrence. Neither time nor authentic samples were available for a very extensive survey of this question. A few experiments, however, were made with the samples of dye-woods available to us. These consisted of logwood, *Hæmatoxylon campechianum;* brazilwood (Pernambuco wood), *Cæsalpina echinata;* hypericum, *Hypericum perforatum;* cam wood, *Baphia nitida;* fustic, *Chlorophora tinctoria* and red saunders, *Pterocarpus santalinus*. Five tests customarily applied to colored woods were employed using the powdered wood itself, cudbear and samples consisting of cudbear containing 25% of the admixed powdered wood. The tests were as follows:

(1) One gram of the comminuted material consisting of woody material or admixtures of woody material and cudbear was macerated in 100 cc. of distilled water for approximately one hour and filtered. The *color* of the filtrate was observed. (The filtrate was used for the following tests.)

(2) Five drops of glacial acetic acid were added to 5 cc. of the filtrate and (A) any color change noted. The mixture was then boiled for one minute and (B) any color change noted. Five drops of stannous chloride T.S. were then added and (C) any color change noted. The mixture was then boiled for one minute and (D) any color change noted.

(3) 2 cc. of basic lead acetate solution were added to 5 cc. of the filtrate and (A) the color change noted. The mixture was then acidified with glacial acetic acid and (B) any subsequent color change noted.

(4) 2 cc. of lime water were added to 5 cc. of the filtrate and the color change noted.

(5) 2 cc. of a 10% aqueous alum solution were added to 5 cc. of the filtrate and the color observed.

The results of the tests will be found in Table V.

COMMENTS ON TABLE V.

A study of Table V shows that while many of the tests are fairly characteristic for the woods themselves, the tests are of doubtful value when the wood is mixed with cudbear. The purplish red coloring principle of cudbear often changes the end color reaction to such an extent as to mask

it entirely or at least to such an extent that it becomes indefinite. It would seem that their histologic characters might be more adaptable to the identification of these woods.

The present N. F. test (our Test No. 2, D) states that logwood and brazilwood give solutions of a deep red color. While this is true for logwood, it is of questionable value for brazilwood. Several samples of brazilwood and Pernambuco wood available to us gave a final light red color alone, and an orange-red color when mixed with cudbear. This final orange-red does not differ enough from the light reddish brown obtained with cudbear to be distinctive.

Attempts to identify the woody material actually occurring in our commercial samples (see Table IV) as one or the other of these colored woods were not successful. The above tests when applied to these samples did not give positive results. As has been previously mentioned it is doubtful whether these colored woods are found as adulterants of cudbear in more than exceedingly rare cases.

All in all, however, it would seem that the question of the identification of the woody adulterants, both the colored woods and others, must be subjected to considerable further investigation before really dependable means for their identification can be established.

TABLE V.—TESTS ON WOODS.

Material.	(1.)	(2.)				(3.)		(4.)	(5.)
		A.	B.	C.	D.	A.	B.		
Cudbear	r. v.	o. r.	r. b.	r. b.	l. r. b.	bl.	r. b.	p.	o. r.
Logwood	d. o. r.	y.	o. y.	d. p.	d. p.	da. bl.	y. b.	p. bla.	d. r.-p.
Brazilwood	o. bf.	y.	y.	l. r.	l. r.	la.	l. y.	r.	y.-r. b.
Hypericum	o. bf.	y.	y.	r.	r.	la.	y.	d. r.	y.-r.
Cam wood	o. y.	y.	y.	o. b.	o. b.	y.	y.	y.	l. y.
Fustic	o.	y.	y.	d. y.	d. y.	y.	g. y.	d. y.	l. g.
Red saunders	l. y. o.	dec.	dec.	dec.	l. r.	dec.	dec.	l. y.	l. y. o.
Cudbear + logwood	d. r.	o.	o.	d. r.	d. r.	bl. r.	b.	p.	r. b.
Cudbear + Brazilwood	r. p.	d. o.	d. o.	o. r.	o. r.	la.	o. r.	p.	r. b.
Cudbear + hypericum	r. p.	o. b.	o. b.	r. b.	r. b.	la.	o.	d. p. r.	r. b.
Cudbear + cam wood	r. p.	o. r.	o.	d. o.	d. o.	la.	o. y.	d. p.	o. r.
Cudbear + fustic	r.	o. r.	o. r.	d. o.	d. o.	r.-bl.	o. r.	d. r.	o. r.
Cudbear + red saund.	p. r.	d. o. r.	d. o. r.	d. o.	d. o.	r. la.	l. o. r.	d. p.	d. o. b.

Explanation of abbreviations: b. brown, bl. blue, bla. black, d. deep, da. dark, dec. decolorized, f. fluorescence, g. green, l. light, la. lavender, o. orange, p. purple, r. red, v. violet, y. yellow.

ARSENIC CONTENT OF CUDBEAR.

Several shipments of cudbear have in the past been denied entry into this country because of high arsenic content. There is no official arsenic standard for cudbear. This work was undertaken to determine the arsenic content of our samples of cudbear.

The samples for the Gutzeit Test were prepared according to the method outlined in A. O. A. C., page 308, part d. (12). After digestion and dilution to a definite volume a small sample was removed and the acidity determined.

The generator used was a modification of that given in Hillebrand and Lundell, page 219 (13). Test-tubes 10 cm. x 1.25 cm. were constricted 6 cm. from one end, and the end then blown out and fire polished, giving a hollow tube constricted 6 cm. from one end. This was fitted to a one-hole rubber stopper, of such size that it fit a 60-cc. wide mouth bottle. The tube for the mercuric bromide paper was 12 cm. long and fitted with a rubber stopper. The lower (longer) portion of the tube contained fluted filter paper 8 cm. x 3 cm. soaked in 20% lead acetate solution and dried. The upper portion contained a roll of the same lead acetate paper well moistened with water and then plugged with cotton and the mercuric bromide tube.

The mercuric bromide strips were strips purchased from A. H. Thomas & Co., and soaked for one hour in 5% alcoholic solution of mercuric bromide, removed and dried.

The test was carried out according to A. O. A. C., page 308, part 4. There agents were all tested and found to be arsenic free. Standards of 0.010, 0.015, 0.020, 0.025 mg. As_2O_3 were run

along with the unknowns each time a determination was made. The lengths of the stains were measured and graphed according to Thomas (14), in which the ratio of the individual length to the total length is plotted against the concentration. This method gives a straight line graph which eliminates the error due to the possibility of several curves being drawn through the same points as when the length is plotted against the concentration. After trial runs to determine the approximate concentration, a sample was chosen which would give a stain within the range of the standards.

Whenever the lower lead acetate paper turned yellow or dark blue the results were not recorded. It was noticed that uniform results could not be obtained when this occurred.

TABLE VI.—ARSENIC CONTENT AS As_2O_3 IN PARTS PER MILLION.

Sample No.	Parts per Million.	Sample No.	Parts per Million.
1	21.0	9	9.3
2	Insufficient for test	10	4325.0
3	13.0	11	93.0
4	1.73	12	2.0
5	35.2	13	1.24
6	78.6	14	Insufficient for test
7	47.7	15	Insufficient for test
8	Insufficient for test	16	0.25

Each result is the average of two or more samples. The accuracy of the Gutzeit Method has been studied by Neller (15) and found to give an average deviation of $\pm 6.6\%$ from the mean of several determinations; approximately the same degree of accuracy is reported by Barnes and Murray (16).

The standard set for certain fruits is not over 1.4 parts As_2O_3 per million. A recent article by Holmes and Remington (17) gives the arsenic content of American Cod Liver Oils as being from 1 to 6 parts per million. Much of the marine life which we use as food contains varying amounts of arsenic. Shrimps contain up to 20 parts of arsenic per million. Norwegians have been taking as much as one ounce of cod liver oil per day without apparent ill effects. If such amounts are harmless in the oils, fruits and marine foods, then in a coloring agent such as cudbear where the actual amount of drug in the finished product is exceedingly small, an arsenic content up to 50 parts per million should be perfectly safe, and possibly justified. It does seem, however, that arsenic is an impurity which enters through carelessness. In a communication from the Department of Agriculture we are advised that six samples received at New York late in 1934 ran 1.2, 1.0, 5.0, 1.0, 2.4 and 1.0 parts per million. It will also be observed that among our own samples several (4, 12, 13, 16) produced in 1933–1934 also ran low in arsenic. This seems to indicate that cudbear with a low arsenic content can be produced if necessary.

The choice of a standard, if it were advisable to establish one, could be based on an amount within the limit of safety as 50 parts per million, but since carelessness appears to be the reason for its presence in large amounts, a maximum of 10 parts per million would be our recommendation. We choose this standard because it would apparently be easy to meet, and would give a coloring agent which would impart practically no arsenic to the finished pharmaceutical preparation. It also corresponds to the arsenic content specified in the present arsenic test of the Pharmacopœia (18).

SUMMARY.

The uniformity, quality and purity of cudbear have been studied. While the commercial samples examined exhibited a surprising uniformity in tinctorial value,

the examination disclosed that several had been intentionally diluted, the most common diluent being potato starch.

(1) A colorimetric method for the determination of quality and a color standard are advised.

(2) Solubility tests are of questionable value in estimating quality.

The discussion of purity involves:

(3) Studies on ash content which reveal that many of the commercial samples run well under the N. F. requirements.

(4) Microscopical studies which indicate considerable adulteration both intentional (the addition of potato starch as a diluent) and unintentional (the presence of woody, bark and leaf tissues).

(5) The usual methods for the detection of dye woods do not give satisfactory results. The presence of dye woods as adulterants of cudbear is, however, probably exceedingly rare.

(6) The study of arsenic content reveals that many samples of cudbear have a considerable amount of arsenic present. Those produced in 1933 and 1934, however, show a smaller content indicating that the presence of arsenic is probably due to carelessness. An arsenic limit of 10 parts per million is tentatively suggested.

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THE DETOXIFICATION OF STRYCHNINE BY PENTOBARBITAL SODIUM.*

BY EDWARD E. SWANSON.[1]

Clinically, Zerfas and McCallum (1) observed that sodium amytal detoxifies strychnine. In animals, Knoefel, Herwick and Loevenhart (2), Dawson and Taft (3) and Haggard and Greenburg (4) reported that several barbituric acid deriva-

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[1] From the Lilly Research Laboratories, Indianapolis.