of the nitrogen monoxide of 0.3 per cent. Therefore, if this assay were made official, hydrogen would have to be recognized as a reagent and a method of determining its purity within 0.1 per cent would be necessary.

SUMMARY.

1. The water solubility method of assay of nitrogen monoxide has been further studied and with certain minor modifications it was found again to yield reasonably accurate and concordant results on standard mixtures of N_2O and N_2.

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DRUG EXTRACTION. XV. A STUDY OF FRACTIONAL PERCOLATION.*[1]

BY WILLIAM J. HUSA[2] AND C. L. HUYCK.

In previous experiments by the present authors it was found that fractional percolation was successful for fluidextract of belladonna root and fairly successful for fluidextract of nux vomica (1).

Further research has been carried out to determine the efficiency of U. S. P. XI fractional percolation and N. F. II fractional percolation as compared with ordinary percolation. The relative efficiency of the different processes was determined by comparing the reserve percolates and finished fluidextracts as to content of alkaloids and extractive matter and by noting the time required in each case. In some instances analyses were also made of various fractions of weak percolate in order to throw further light on the progress of the extraction at various stages of fractional percolation.

HISTORICAL REVIEW.

In 1833, M. Boullay (2) of France suggested an extraction process for difficultly extractable substances, called "continuous displacement," in which the drug was divided in several containers and the liquid allowed to pass successively from one to another. E. R. Squibb (3) modified this process in 1866 by collecting a reserve percolate and several fractions of weak percolate from each portion of drug. At first Squibb called this method "divided percolation," but in 1867 he introduced the term "repercolation" (4). The name "fractional percolation" was applied by Diehl (5) to a repercolation process in which no weak percolate was left when the fluidextract was completed.

The greatest disadvantage of Squibb's repercolation process was that when a fluidextract was prepared some weak percolate remained which had to be kept in storage until the next time the fluidextract was made. After further experiments, notably by Squibb (6), Diehl (5), Lloyd (7) and Hallberg (8), a fractional percola-

* Scientific Section, A. PH. A., New York meeting, 1937.

[1] This paper is based on part of a dissertation presented to the Graduate Council of the University of Florida by C. L. Huyck, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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tion process was included in the first edition of the National Formulary in 1888. The N. F. process divided 16 troy ounces of drug into three portions of 8, 5 and 3 troy ounces, respectively, and no weak percolate was collected from the last portion. The later fractional percolation processes which have been official in the U. S. P. and N. F. have all been of the same type as that of the N. F. I, with only minor changes in details.

EXPERIMENTAL PART.

Materials Used.—To insure uniformity throughout the experimental work all of the drug used was from a thoroughly mixed 100-lb. portion of moderately coarsely powdered belladonna root. The menstruum used was a mixture of four volumes of alcohol and one volume of distilled water as specified for fluidextract of belladonna root in the U. S. P. XI.

Methods of Analysis.—Alkaloids were determined by the U. S. P. XI assay process for fluidextract of belladonna root. To determine total extractive, ten cc. of the liquid was evaporated to dryness on a water-bath and then heated in an oven at 105° C. until the difference between two successive weighings did not exceed ten mg.

Preparation of Fluidextract of Belladonna Root by Percolation.—Two separate 1000-cc. portions of fluidextract of belladonna root were made by ordinary percolation. In each case a 1000-Gm. portion of drug moistened with 600 cc. of menstruum was placed in a percolator in about five portions with agitation after each addition to promote even distribution, and after all the drug had been thus introduced it was packed down from the top, using a wooden potato masher and starting with light pressure which was gradually increased.

After macerating for forty-eight hours the percolate was allowed to flow at a rate of two cc. per minute, a reserve portion of 800 cc. and 2200 cc. of weak percolate being collected in each case. The weak percolate was placed in a vacuum distillation apparatus and evaporated at a temperature not exceeding 60° C. In the case of the fluidextract designated as "A," the vacuum distillation was continued until bubbles no longer were formed on the surface of the residue; with fluidextract "B" vacuum distillation was continued until no more distillate came through the condenser. As a result the residue in A was a soft extract which dissolved readily in the slightly warmed reserve portion while that from B was a much firmer extract which dissolved with difficulty. Fluidextract B showed much more precipitation than fluidextract A after storing one week.

Other experimental details were as follows: dimensions of percolators, length 56.5 cm.; internal diameter at the top, 11.3 cm.; length of drug column, 37 cm.; time of operator required for each portion of fluidextract, 4.2 hours; total elapsed time, 108.5 hours.

Analytical data on the finished fluidextracts are given in Table I.

TABLE I.—ASSAY RESULTS ON FLUIDEXTRACTS OF BELLADONNA ROOT MADE BY ORDINARY PERCOLATION.

	Gm. Alkaloids in 1000 Cc.	Gm. Total Extractive in 1000 Cc.
Fluidextract A	5.9	168.4
Fluidextract B	5.7	172.5
Average	5.8	170.4

Preparation of Fluidextract of Belladonna Root by U. S. P. XI Fractional Percolation.—Four different 1000-cc. portions of fluidextract of belladonna root were prepared by U. S. P. XI fractional percolation, using two different methods of packing. In the method of packing designated as "from top" the moistened drug was introduced into the percolator in small portions with slight agitation of the percolator to promote even distribution, and after all the drug had been thus introduced it was packed down from the top, using a wooden potato masher and starting with light pressure which was gradually increased. In the method of packing designated as "in sections," the drug was introduced in about eight portions and each separate portion packed down. The method of packing in sections gave tighter packing as evidenced by the smaller volume of packed drug. The proportion of menstruum used for moistening was 60 cc. per 100 Gm. of drug. The rate of percolation was 2 cc. per minute. The U. S. P. XI fractional percolation process was

followed with the exception that the final reserve portion of 500 cc. was collected in successive fractions of 300 cc. and 200 cc., respectively, in order to throw more light on the progress of extraction in the final stages.

Experimental details and analytical data are given in Table II. Of the four experiments, C and D were carried out simultaneously as a first series, and E and F made up a later series. The results of Experiments C and D indicated that the fluidextract prepared from the drug packed in sections contained slightly more alkaloids and extractive matter than the fluidextract prepared from the drug packed from the top. Packing from the top gave a larger volume of packed drug than packing in sections, but in the latter case the length of the drug column was greater because smaller percolators were used.

It seemed desirable to carry out another similar experiment in which the two different methods of packing would be compared using corresponding percolators of the same size. Accordingly, experiments E and F were carried out, using corresponding percolators of the same size for each method of packing. Under these conditions the greater drug volume obtained in packing from the top gave a longer drug column than was obtained by packing in sections.

The data in Table II indicate that the fluidextracts made by the two methods of packing in corresponding percolators of the same size were practically identical. Hence there seems to be no particular advantage in either method of packing in fractional percolation of belladonna root.

TABLE II.—PREPARATION OF FLUIDEXTRACT OF BELLADONNA ROOT BY U. S. P. XI FRACTIONAL PERCOLATION.

	Exp. C Packed from Top.	Exp. D Packed in Sections.	Exp. E Packed from Top.	Exp. F Packed in Sections.
Inside Dimensions of the Percolators in Gm.				
For 500-Gm. portion				
Length	42.5	36.5	42.0	43.0
Width (at top)	8.5	7.3	8.5	8.7
For 300-Gm. portion				
Length	36.5	29.5	37.0	36.3
Width (at top)	7.5	5.8	7.9	7.5
For 200-Gm. portion				
Length	28.5	27.5	29.0	29.0
Width (at top)	5.5	5.8	5.8	5.5
Volume of Packed Drug in Cc.				
500-Gm. portion	1325	1125	1300	1100
300-Gm. portion	750	675	750	650
200-Gm. portion	530	450	475	430
Total	2605	2250	2525	2180
Length of Drug Column in Gm.				
500-Gm. portion	26.0	27.0	27.5	25.0
300-Gm. portion	17.0	22.5	18.5	16.5
200-Gm. portion	21.0	17.5	19.0	18.5
Total	64.0	67.0	65.0	60.0
Average temperature during experiment	25° C.	25° C.	24° C.	24° C.
Time of operator required, in hours	2.2	2.9	2.1	2.7
Total elapsed time, in hours	176	176	176	176
Total Alkaloids in Various Fractions of Percolate, in Gm.				
First reserve—200 cc.	1.3	1.5	1.6	1.5
Second reserve—300 cc.	2.4	2.6	2.6	2.5
Third reserve				
First portion—300 cc.	1.5	1.3	1.6	1.8
Second portion—200 cc.	0.1	0.1	0.1	0.1
Total—1000 cc.	5.3	5.5	5.9	5.9

Total Extractive in Various Fractions of Percolate, in Gm.				
First reserve—200 cc.	20.1	19.8	19.0	20.2
Second reserve—300 cc.	39.3	40.1	37.4	37.4
Third reserve				
First portion—300 cc.	42.3	44.4	39.3	38.4
Second portion—200 cc.	18.9	20.4	21.3	19.4
Total—1000 cc.	120.6	124.7	117.0	115.4

Preparation of Fluidextract of Belladonna Root by N. F. II Fractional Percolation.—In the N. F. II fractional percolation method the drug and reserve percolate are divided into portions of 500, 325 and 175 Gm. and cc., respectively, as compared with 500, 300 and 200 Gm. or cc. in the U. S. P. XI process. Another difference is that the quantity of weak percolate collected from the second portion of drug is 650 cc. in the N. F. II as compared with 1000 cc. in the U. S. P. XI.

Fluidextract of belladonna root was prepared from two 1000-Gm. portions of drug, using the two different methods of packing previously described. The proportion of moistening liquid was 60 cc. per 100 Gm. of drug and the rate of flow was 2 cc. per minute.

The dimensions of the percolators used in Exp. G were the same as in Exp. C while those in Exp. H were the same as in Exp. D (see Table II). The temperature was approximately 25° C. The time of an operator required was 1.6 hours for Exp. G and 2 hours for Exp. H; the total elapsed time in each case was 176 hours. Further data are given in Table III.

TABLE III.—PREPARATION OF FLUIDEXTRACT OF BELLADONNA ROOT BY N. F. II FRACTIONAL PERCOLATION.

	Volume of Packed Drug in Cc.		Length of Drug Column in Cc.	
	Exp. G Packed from Top.	Exp. H Packed in Sections.	Exp. G Packed from Top.	Exp. H Packed in Sections.
500-Gm. portion	1300	1125	27.5	29.0
325-Gm. portion	875	750	22.0	27.5
175-Gm. portion	465	385	20.0	17.5
Total	2640	2260	69.5	74.0
	Gm. Alkaloids in Percolates.		Gm. Total Extractive in Percolates.	
First reserve—175 cc.	1.3	1.3	19.0	19.7
Second reserve—325 cc.	2.4	2.9	44.6	44.5
Third reserve				
First portion—300 cc.	1.5	1.3	43.7	42.0
Second portion—200 cc.	0.2	0.2	19.1	19.4
Total—1000 cc.	5.4	5.7	126.4	125.6

Within experimental error, the fluidextracts of belladonna root made by N. F. II fractional percolation contained the same proportion of alkaloids and extractive matter as were present in the fluidextracts made by U. S. P. XI fractional percolation. Apparently the differences in proportions of drug and reserve percolate in the two processes have no appreciable effect on the efficiency of extraction.

Analysis of the Repercolation Process.—In order to throw further light on the progress of extraction at various stages of fractional percolation, experiments were conducted in which analyses were made of the different fractions of reserve and weak percolate. Fractional percolation was conducted according to the U. S. P. XI directions, with the exception that the first portion of drug was doubled and the third portion reduced by one-half, in order that one-half of each fraction of weak percolate could be set aside for analysis. In addition to this, a 300-Gm. portion of drug was percolated with fresh menstruum, a reserve percolate of 300 cc. and five successive 200-cc. fractions of weak percolate being collected; by analyzing these percolates and comparing them with the percolates obtained from a 300-Gm. portion of drug percolated with weak percolate, it was possible to draw a conclusion as to whether weak percolate is a better solvent for the

active constituents than the original menstruum. Each percolation and each analysis was carried out in duplicate, the data in Tables IV–VI, inclusive, being averages.

Each portion of drug was moistened, allowed to macerate for fifteen minutes and packed from the top. The moistening liquid was used in the proportion of 25 cc. of liquid to 100 Gm. of drug. The rates of flow in fractional percolation were 3.5 cc. per minute for the 1000-Gm. portions of drug, 1.8 cc. per minute for the 300-Gm. portions and 1.3 cc. per minute for the 100-Gm. portions. For the 300-Gm. portion of drug percolated with fresh menstruum the rate of flow was 2.5 cc. per minute.

The finished fluidextracts made by fractional percolation (Exps. I and J) contained 5.7 Gm. of alkaloids and 149 Gm. of total extractive in 1000 cc. of fluidextract. The weights of alkaloids and total extractive in the various fractions of percolate were recalculated on a percentage basis, taking the quantities present in the finished fluidextracts as 100%. The figures thus obtained show the percentage of total extracted alkaloids and extractive present in each fraction of reserve and weak percolate; these data are presented in Table V.

The data in Tables IV and V indicate the rate at which the extraction of belladonna root proceeds at various stages of fractional percolation. The reserve percolates from the first two portions of drug contain a large proportion of the alkaloids present but complete exhaustion of the alkaloids in the drug proceeds slowly after that, since even the fifth portion of weak percolate contains about one or two per cent of the total alkaloids present in the three portions of drug taken as a whole.

TABLE IV.—ANALYSES OF ALL FRACTIONS OF PERCOLATE IN PREPARATION OF FLUIDEXTRACT OF BELLADONNA ROOT BY U. S. P. XI FRACTIONAL PERCOLATION.

From 1000-Gm. Portion of Drug.	Gm. Alkaloids in Fraction of Percolate.	Gm. Total Extractive in Fraction of Percolate.
Reserve—400 cc.	4.31	63.9
Weak percolate—600 cc.	0.81	73.4
Weak percolate—600 cc.	0.26	46.4
Weak percolate—600 cc.	0.20	21.1
Weak percolate—600 cc.	0.19	6.2
Weak percolate—600 cc.	0.23	3.5
Total	6.00	214.5
From 300-Gm. Portion of Drug.		
Reserve—300 cc.	1.92	51.8
Weak percolate—200 cc.	0.20	23.4
Weak percolate—200 cc.	0.14	17.6
Weak percolate—200 cc.	0.08	13.6
Weak percolate—200 cc.	0.07	10.2
Weak percolate—200 cc.	0.04	6.9
Total	2.45	123.5
From 100-Gm. Portion of Drug.		
Reserve—250 cc.	0.81	37.2

TABLE V.—PERCENTAGE OF TOTAL EXTRACTED ALKALOIDS AND EXTRACTIVE PRESENT IN VARIOUS FRACTIONS OF PERCOLATE.

First Portion—500 Gm.	Per Cent of Total Alkaloids.	Per Cent of Total Extractive.
Reserve—200 cc.	37.9	20.2
Weak percolate—300 cc.	7.1	23.2
Weak percolate—300 cc.	2.3	14.7
Weak percolate—300 cc.	1.8	6.7
Weak percolate—300 cc.	1.8	2.0
Weak percolate—300 cc.	2.0	1.1

Second Portion—300 Gm.		
Reserve—300 cc.	33.7	32.8
Weak percolate—200 cc.	3.5	14.8
Weak percolate—200 cc.	2.5	11.1
Weak percolate—200 cc.	1.4	8.6
Weak percolate—200 cc.	1.2	6.4
Weak percolate—200 cc.	0.7	4.4
Third Portion—200 Gm.		
Reserve—500 cc.	28.4	47.0

TABLE VI.—COMPARISON OF THE EXTRACTION OF 300-GM. PORTIONS OF BELLADONNA ROOT WITH FRESH MENSTRUUM AND WEAK PERCOLATE.

	Gm. Extracted Substances in Various Fractions of Percolate.			
	Alkaloids.		Total Extractive.	
	Fresh* Menstruum.	Weak* Percolate.	Fresh* Menstruum.	Weak* Percolate.
Reserve—300 cc.	1.81	1.92	40.7	51.8
Weak percolate—200 cc.	0.10	0.20	15.4	23.4
Weak percolate—200 cc.	0.05	0.14	6.2	17.6
Weak percolate—200 cc.	0.03	0.08	2.3	13.6
Weak percolate—200 cc.	0.03	0.07	1.3	10.2
Weak percolate—200 cc.	0.03	0.04	1.0	6.9
Total	2.05	2.45	66.9	123.5

* Menstruum used.

According to Bennett and Cocking (9) a weak solution of the extractive of a drug is usually a better solvent for the active constituents than the original menstruum. From the data in Table VI and considering the quantity of alkaloids and extractive matter already present in the weak percolate from the first portion of drug (see Table IV) this statement does not appear to be true in the extraction of belladonna root. For this drug, at least, the only advantage in using weak percolate in fractional percolation is simply that a stronger percolate is obtained by using as menstruum a liquid which is already partly saturated with drug constituents.

DISCUSSION OF RESULTS.

Method of Packing.—In an earlier paper by the present authors (10) it was shown that packing the drug from the top was somewhat better than packing in sections in the percolation of belladonna root. In citing this paper, Büchi and Feinstein (11) stated that this was the first accurate study of efficiency of different methods of packing that was published in more than a century of percolation history. The conclusions reached by the present authors on belladonna root were verified in the case of cinchona by Büchi and Feinstein (11). Although there was a slight but definite difference in favor of packing from the top in simple percolation of both belladonna root and cinchona, the present investigation has shown that in repercolation of belladonna root the method of packing has no appreciable influence on the efficiency of extraction. In this connection it may be noted that the earlier study (10) showed that packing from the top was particularly advantageous in fast percolation conducted without maceration, the difference being much smaller when there was a forty-eight hour period of maceration after packing, as was the case in repercolation in the present study.

As may be seen in Table II, the volume of the packed drug was about ten per cent higher when packed from the top than when packed in sections. Büchi and Feinstein showed that lightly packed cinchona absorbed more menstruum than the

tightly packed drug. Likewise it was noted in the present study that more menstruum was used when the drug was packed from the top, this being due to the greater amount of menstruum remaining between the particles of the more lightly packed drug.

In making a 1000-cc. portion of fluidextract, the method of packing in sections required about 40 minutes longer than packing from the top.

Packing from the top is therefore advantageous in saving time of packing, and gives somewhat more efficient extraction in rapid percolation without maceration while packing in sections has the advantage of leaving less solvent to be recovered from the marc.

Comparison of Simple Percolation and Fractional Percolation.—The U. S. P. allows the use of Process C as an alternative for Processes A or B.

TABLE VII.—COMPARISON OF FLUIDEXTRACTS OF BELLADONNA ROOT MADE BY PROCESSES A AND C.

	Gm. Alkaloids in 1000 Cc.	Gm. Total Extractive in 1000 Cc.	Time of Operator Required in Hours.	Elapsed Time Required in Hours.
Process A (Percolation)	5.8	170.4	4.2	108½
Process C (Fractional percolation)	5.7	119.4	2.2	176

Comparative data on fluidextracts of belladonna root made by Processes A and C are given in Table VII. The fluidextract made by Process C contains about the same proportion of alkaloids as that made by Process A. Simple percolation, however, yields a fluidextract containing about forty per cent more total extractive than is obtained by fractional percolation. The greatly increased proportion of total extractive obtained by Process A is due to retention of much of the extractive matter present in the large proportion of weak percolate which is evaporated to a soft extract and dissolved in the reserve percolate. The difference in content of total extractive doubtless also causes a difference in alcohol contents of the fluidextracts; possibly this point has not received sufficient consideration in the past in setting official standards for alcohol content of fluidextracts.

Fractional percolation requires the packing of more percolators and the collection of more separate percolates than simple percolation but regardless of this it was found that Process A required almost twice as much of the operator's time as Process C, largely because of the time required in Process A for attention to the evaporation of the weak percolate *in vacuo* and the handling of the soft extract. On the other hand, the elapsed time required in fractional percolation was much greater, chiefly because of the time required for maceration and percolation of three portions of drug as compared with one portion in ordinary percolation.

Effect of Proportion of Moistening Liquid.—Husa and Yates (12) found that in simple percolation of belladonna root the efficiency of extraction was promoted by using a low proportion of menstruum in moistening the drug for preliminary maceration. In the present study, further data have been obtained in applying this principle to repercolation, the fluidextracts being prepared in 1000-cc. quantities.

TABLE VIII.—COMPARISON OF FLUIDEXTRACTS OF BELLADONNA ROOT MADE WITH DIFFERENT PROPORTIONS OF MOISTENING LIQUID.

	Gm. Alkaloids in Various Fractions of Percolate.		Gm. Total Extractive in Various Fractions of Percolate.	
	60 Cc.*	25 Cc.*	60 Cc.*	25 Cc.*
First reserve—200 cc.	1.6	2.2	19.0	31.9
Second reserve—300 cc.	2.6	1.9	37.4	51.7
Third reserve—500 cc.	1.7	1.6	60.6	74.2
Total—1000 cc.	5.9	5.7	117.0	157.8

* Quantity of menstruum used per 100 Gm. of drug for preliminary maceration.

From the data in Table VIII it is seen that the quantity of alkaloids in the first reserve is much greater when a smaller proportion of moistening liquid is used. In the second reserves the situation is reversed because the quantity of alkaloids which failed to reach the first reserve when more moistening liquid was used now is found in the second reserve. In the case of the alkaloids the advantage gained in the first reserve was lost by the time all three reserves had been collected. As to extractive matter, however, the use of a smaller proportion of moistening liquid gave more total extractive in each of the three reserves.

In securing efficient extraction of alkaloids, the use of only a small proportion of moistening liquid is not as advantageous as it is in simple percolation. However, the fact that a smaller proportion of moistening liquid gave a finished fluidextract containing considerably more total extractive than that made with more moistening liquid presents a problem that requires consideration. Whether the higher content of total extractive is desirable or undesirable is a debatable question. On the other hand it is clearly evident that fluidextracts made by different operators will vary considerably in alcohol content, specific gravity and content of total extractive unless the proportion of moistening liquid used is more rigidly standardized.

Proportion of Weak Percolate Collected in Fractional Percolation.—The various editions of the U. S. P. and N. F. have differed somewhat in the directions regarding the quantity of weak percolate to be collected from the second portion of drug in fractional percolation. At present both the U. S. P. XI and N. F. VI direct that 1000 cc. of weak percolate in five successive fractions of 200 cc. each shall be collected from the second portion of drug when making 1000 cc. of finished fluidextract. However, the U. S. P. VIII and IX required the collection of 800 cc. of weak percolate at this point, while the N. F. II and III required 650 cc.; in the N. F. IV and V the quantity was not definitely specified.

In seeking to specify a definite quantity of weak percolate there are obviously two opposing factors to be balanced. If the quantity of weak percolate from the second portion is too large, some of it will remain unused when the final reserve has been collected. If the quantity of weak percolate is too small at this point, it is necessary to use some fresh menstruum and the extractive value which this quantity of menstruum might have exerted on the second portion of drug is lost. Varying opinions as to how these two factors should be balanced doubtless account for the seeming vacillation in official standards on this point.

In the present study it was observed that when 1000 cc. of weak percolate is collected from the second portion of belladonna root, there is always a remainder of weak percolate when the third reserve has been collected; the portion remaining ranges from 150 to 250 cc. when making 1000 cc. of fluidextract. When 800 cc. of weak percolate is collected in accordance with the U. S. P. X directions, it was observed by the present authors in a previous investigation (13) that in making fluidextracts of belladonna root, nux vomica and cinchona there was a remainder of 40 to 55 cc. of weak percolate at the end of the process. In the present study it was found that when 650 cc. of weak percolate was collected from the second portion of belladonna root, as was directed by the N. F. II, more than 100 cc. of fresh menstruum was required to secure the full amount of reserve from the third portion of drug.

On the basis of our studies on belladonna root, nux vomica and cinchona, it is concluded that the collection of 800 cc. of weak percolate from the second portion of drug as specified in the U. S. P. X is satisfactory and that this proportion is preferable to that of the U. S. P. XI and N. F. VI, as well as to that of the N. F. II and III. The extra 200 cc. collected at present is of no value, since its content of dissolved substances does not appear in the finished fluidextract. On the other hand, the N. F. II and III did not make full use of the solvent power of all the menstruum used.

SUMMARY.

Fluidextract of belladonna root made by fractional percolation contains about the same proportion of alkaloids as that made by ordinary percolation but the latter contains a much higher proportion of total extractive matter. The preparation of a fluidextract by fractional percolation as compared with ordinary percolation requires less time of an operator but the total elapsed time is greater. Packing from the top is advantageous in saving time of packing, while packing in sections has the advantage of leaving less solvent to be recovered from the marc. Data are

presented showing the content of alkaloids and total extractive in every fraction of reserve and weak percolate collected in fractional percolation of belladonna root.

In experiments in which the proportion of moistening liquid was varied it was found that the use of a smaller proportion of moistening liquid gave a finished fluidextract containing considerably more total extractive. It is apparent that fluidextracts made by different operators will vary considerably unless the proportion of moistening liquid is more rigidly standardized.

On the basis of studies on belladonna root, nux vomica and cinchona, it is concluded that in fractional percolation the collection of 800 cc. of weak percolate from the second portion of drug as specified in the U. S. P. X is satisfactory and that this proportion is preferable to that of the U. S. P. XI and N. F. VI, as well as to that of the N. F. II and III.

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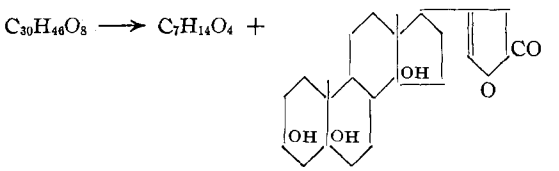
THE POTENCY OF PERIPLOCYMARIN, BUFOTALIN AND DESACETYL-OLEANDRIN.*

BY K. K. CHEN, ROBERT C. ANDERSON AND E. BROWN ROBBINS.[1]

Jacobs and Hoffmann (1) in 1928 isolated a cardiac monoside, periplocymarin, from the stems of *Periploca græca*, by his ingenious method of enzymatic digestion. Structural studies were carried out during subsequent years by Jacobs and Elderfield (2) and Elderfield and Rothen (3) until the following formula has been established (4):



Periplocymarin Cymarose Periplogenin

In 1913 Wieland and Weil (5) succeeded in crystallizing a cardiac principle, bufotalin, from the skin of the common European toad, *Bufo vulgaris* (*Bufo bufo bufo*), a substance resembling Abel's bufagin (6) but not identical with it. The constitution of bufotalin was repeatedly illucidated by Wieland (7), Wieland and Alles (8), Wieland, Hesse and Meyer (9), and Wieland and Hesse (10). A structural formula was finally proposed by Wieland, Hesse and Hüttel (11). As shown on page 114, it is

* Scientific Section, A. PH. A., New York meeting, 1937.

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