4. No antipyretic effect was noted after administration of the drug to rabbits.

5. The drug paralyzed smooth muscle preparations *in vitro* and had no truly laxative effect on the intestine *in vivo*.

6. Intravenous injection of saline suspensions of *Phytolacca* in cats produced marked depression and paralysis of both circulation and respiration.

7. These findings substantiate none of the claims made for the drug in the old textbooks.

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THE USE OF NEW SOLVENTS IN ALKALOIDAL ASSAYS.*

BY M. L. JACOBS [1] AND GLENN L. JENKINS.[2]

I. SOLUBILITIES AND DISTRIBUTION COEFFICIENTS OF CERTAIN ALKALOIDS IN ISOPROPYL ETHER AND METHYLENE CHLORIDE.[3]

SOLUBILITIES.

The solubilities of strychnine, quinine, atropine and caffeine in isopropyl ether, methylene chloride, mixtures of isopropyl ether-methylene chloride, mixtures of ethyl ether-chloroform, mixtures of isopropyl ether-chloroform and mixtures of ethyl ether-methylene chloride were determined.

The method employed for these determinations was as follows:

Twenty-five cc. of the solvent were placed in a small bottle and enough of the alkaloid added to insure an excess after shaking in a mechanical shaker over night. The bottle was then placed in a thermostat bath, regulated at 25° C. to 0.1°, and allowed to remain in the bath for at least twelve hours in order that equilibrium between the solute and solvent would be reached. A volume of about 5 cc. was then pipetted off, placed in a tared weighing bottle and its weight recorded. The solvent was allowed to evaporate spontaneously, the residue dried to constant weight at 100° C., cooled in a desiccator over sulfuric acid and its weight recorded.

The bottle was again shaken for three hours in a mechanical shaker and a sample determined as before. This procedure was repeated until constant results were obtained which was usually after the second shaking.

The calculations were made upon a basis of grams of alkaloid soluble in 100 Gm. of solvent at 25° C. and in the cases of chloroform and ether the solubilities of the various alkaloids were calculated from solubility data given in the United States Pharmacopœia, X.

The accompanying tables (Tables I, II, III and IV) show clearly the solubilities of the above-mentioned alkaloids in the individual solvents and mixed solvents under consideration.

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TABLE I.—STRYCHNINE.

Grams Soluble in 100-Gm. Solvent.

Ethyl ether	Ethyl ether, 3 vol. Chloroform, 1 vol.	Ethyl ether, 1 vol. Chloroform, 1 vol.	Ethyl ether, 1 vol. Chloroform, 3 vol.	Chloroform
0.021	1.62	3.26	6.27	13.5
Isopropyl ether	Isopropyl ether, 3 vol. Chloroform, 1 vol.	Isopropyl ether, 1 vol. Chloroform, 1 vol.	Isopropyl ether, 1 vol. Chloroform, 3 vol.	Chloroform
0.058	1.73	3.38	7.39	13.5
Isopropyl ether	Isopropyl ether, 3 vol. Methylene chlor., 1 vol.	Isopropyl ether, 1 vol. Methylene chlor., 1 vol.	Isopropyl ether, 1 vol. Methylene chlor., 3 vol.	Methylene Chloride
0.058	0.061	0.068	0.077	0.08
Ethyl ether	Ethyl ether, 3 vol. Methylene chlor., 1 vol.	Ethyl ether, 1 vol. Methylene chlor., 1 vol.	Ethyl ether, 1 vol. Methylene chlor., 3 vol.	Methylene Chloride
0.021	0.029	0.032	0.047	0.08

TABLE II.—QUININE.

Grams Soluble in 100-Gm. Solvent.

Ethyl ether	Ethyl ether, 3 vol. Chloroform, 1 vol.	Ethyl ether, 1 vol. Chloroform, 1 vol.	Ethyl ether, 1 vol. Chloroform, 3 vol.	Chloroform
74.00			44.31	61.7
Isopropyl ether	Isopropyl ether, 3 vol. Chloroform, 1 vol.	Isopropyl ether, 1 vol. Chloroform, 1 vol.	Isopropyl ether, 1 vol. Chloroform, 3 vol.	Chloroform
0.312			37.9	61.7
Ethyl ether	Ethyl ether, 3 vol. Methylene chlor., 1 vol.	Ethyl ether, 1 vol. Methylene chlor., 1 vol.	Ethyl ether, 1 vol. Methylene chlor., 3 vol.	Methylene Chloride
74.00			41.80	58.2
Isopropyl ether	Isopropyl ether, 3 vol. Methylene chlor., 1 vol.	Isopropyl ether, 1 vol. Methylene chlor., 1 vol.	Isopropyl ether, 1 vol. Methylene chlor., 3 vol.	Methylene Chloride
0.312			40.84	58.2

TABLE III.—CAFFEINE.

Grams Soluble in 100-Gm. Solvent.

Ethyl ether	Ethyl ether, 3 vol. Chloroform, 1 vol.	Ethyl ether, 1 vol. Chloroform, 1 vol.	Ethyl ether, 1 vol. Chloroform, 3 vol.	Chloroform
0.18	0.39	1.58	5.39	12.20
Isopropyl ether	Isopropyl ether, 3 vol. Chloroform, 1 vol.	Isopropyl ether, 1 vol. Chloroform, 1 vol.	Isopropyl ether, 1 vol. Chloroform, 3 vol.	Chloroform
0.15	0.30	1.39	5.29	12.20
Isopropyl ether	Isopropyl ether, 3 vol. Methylene chlor., 1 vol.	Isopropyl ether, 1 vol. Methylene chlor., 1 vol.	Isopropyl ether, 1 vol. Methylene chlor., 3 vol.	Methylene Chloride
0.15	0.29	1.27	4.50	8.67
Ethyl ether	Ethyl ether, 3 vol. Methylene chlor., 1 vol.	Ethyl ether, 1 vol. Methylene chlor., 1 vol.	Ethyl ether, 1 vol. Methylene chlor., 3 vol.	Methylene Chloride
0.18	0.26	1.97	4.72	8.67

TABLE IV.—ATROPINE.

Grams Soluble in 100-Gm. Solvent.

Ethyl ether	Ethyl ether, 3 vol. Chloroform, 1 vol.	Ethyl ether, 1 vol. Chloroform, 1 vol.	Ethyl ether, 1 vol. Chloroform, 3 vol.	Chloroform
5.63	12.14	28.50	52.50	67.56
Isopropyl ether	Isopropyl ether, 3 vol. Chloroform, 1 vol.	Isopropyl ether, 1 vol. Chloroform, 1 vol.	Isopropyl ether, 1 vol. Chloroform, 3 vol.	Chloroform
1.03	11.22	24.9	47.20	67.56
Ethyl ether	Ethyl ether, 3 vol. Methylene chlor., 1 vol.	Ethyl ether, 1 vol. Methylene chlor., 1 vol.	Ethyl ether, 1 vol. Methylene chlor., 3 vol.	Methylene Chloride
5.63	17.69	32.23	45.55	65.23
Isopropyl ether	Isopropyl ether, 3 vol. Methylene chlor., 1 vol.	Isopropyl ether, 1 vol. Methylene chlor., 1 vol.	Isopropyl ether, 1 vol. Methylene chlor., 3 vol.	Methylene Chloride
1.03	10.08	22.9	43.7	65.23

It will be observed upon examination of Table I that strychnine is more soluble in isopropyl ether than in ethyl ether, and a great deal more soluble in chloroform than in methylene chloride. The best solvent, then, for strychnine is chloroform and the best mixed solvent is a 3:1 mixture of isopropyl ether and chloroform.

Table II indicates that quinine is a great deal more soluble in ethyl ether than in isopropyl ether, while its solubility in methylene chloride is slightly less than in chloroform. Due to a gradual darkening and the formation of a viscid liquid in each case, it was very difficult to prepare saturated solutions of quinine in methylene chloride and in mixed solvents where methylene chloride was one of the components. It would appear, however, that quinine is not nearly as soluble in mixtures of ethyl ether-methylene chloride and isopropyl ether-methylene chloride as might be expected when its solubility in the individual solvents is considered. It is also of interest to note that quinine in the presence of methylene chloride gradually undergoes decomposition. Such a decomposition, however, would probably not interfere with the use of this solvent in the assay of cinchona, since the assay could be completed before any appreciable change took place. Experiments will be recorded later to substantiate or disprove this conclusion.

Table III shows that caffeine is soluble to the extent of 0.18 Gm. in 100 Gm. of ethyl ether and 0.15 Gm. in 100 Gm. of isopropyl ether. It also shows that caffeine is soluble in chloroform to the extent of 12.2 Gm. per 100 Gm. of solvent and in methylene chloride to the extent of 8.67 Gm. in 100 Gm. of solvent. In other words, isopropyl ether is practically as good a solvent as ethyl ether for caffeine, while methylene chloride dissolves only about two-thirds as much as chloroform.

In Table IV it may be seen that atropine is soluble in ethyl ether to the extent of 5.63 Gm. per 100 Gm. of solvent and in isopropyl ether to the extent of 1.03 Gm. per 100 Gm. of solvent. Its solubility in chloroform is 67.56 Gm. per 100 Gm. of solvent, and in methylene chloride 65.23 Gm. per 100 Gm. of solvent.

DISTRIBUTION COEFFICIENTS.

In 1914, Marden and Elliott (1) published a paper on the methods of extraction by means of immiscible solvents, in which they dealt mainly with distribution ratios of certain alkaloids between water and the immiscible solvents, chloroform and ether. These investigators pointed out that by use of the distribution coefficients, and a certain algebraic formula, the number of extractions necessary to remove practically all of a given alkaloid from aqueous solution could be calculated.

To calculate the distribution ratio (d) of the various alkaloids they used the following expression:

$$\frac{\text{Concentration in 10 cc. water}}{\text{Concentration in 10 cc. of non-aqueous solvent}} = \frac{C_1}{C_2} = (d)$$

and for the calculation of the number of shakings necessary for an extraction the following algebraic formula:

$$\frac{X_n}{X_o} = \left(\frac{da}{e + da}\right)^n$$

where

a = volume of aqueous solvent
e = volume of non-aqueous solvent

X_o = original amount of material in the aqueous layer to be extracted
X_n = amount of material in the water layer after n extractions.

The expression X_n/X_o = the fraction of material in the water layer after n extractions. Thus, it is seen that the smaller the value of X_n/X_o when calculated from the above formula, the fewer number of extractions necessary for complete removal of the alkaloid from aqueous solution.

In the aconitine, ether and aqueous ammoniacal system, using 100 cc. of water, 5 cc. of ammonia water and 50 cc. of ether, the result, (d) = 0.140 was obtained, and when 30 cc. of chloroform was used in place of the 50 cc. of ether the value of 0.017 (d = 0.017) was obtained. Thus, chloroform is shown to be a better immiscible solvent than ether for extracting aconitine from aqueous solution.

In the system atropine, water and chloroform the distribution ratio was found to be small, therefore, three extractions with 10-cc. portions of chloroform from 50 cc. of aqueous solution completely removed the atropine.

Codeine showed a high distribution ratio between water and ether (d = 0.939) and a low ratio between water and chloroform (d = 0.0067), indicating that chloroform is much the better immiscible solvent. Experiments showed this to be true.

The value of (d) for coniine between water and ether was shown to be about 0.05 and it was found that from three to five extractions, with 10-cc. portions of ether, would extract more than 99 per cent of this alkaloid. It was pointed out that due to the volatility of the coniine the partition ratio was hard to obtain.

The distribution ratio of quinine between water and chloroform was found to be very small. Thus, three washings with 10-cc. portions of chloroform almost completely removed the quinine.

The value of knowing the distribution ratio between immiscible solvents in alkaloidal assaying is apparent when one considers that from such knowledge the number of extractions necessary in any given case may be calculated. Therefore, the tedious process of testing for complete extraction by means of some alkaloidal reagent is eliminated. With this thought in mind it was decided to determine the distribution ratios of certain alkaloids in the systems water–isopropyl ether, water–methylene chloride, and also, between water and various mixtures of these immiscible solvents. With this data at hand it was thought that a more intelligent study of the use of these solvents in alkaloidal assaying could be made.

Accordingly, the distribution ratios of atropine, caffeine, quinine and strychnine between water and isopropyl ether, water and methylene chloride and water and mixtures of isopropyl ether-methylene chloride were determined.

In calculating the distribution ratios (d) the solubility of methylene chloride in water and the solubility of water in methylene chloride was considered to be negligible, therefore, the volume of each upon saturation with the other was taken as the initial volume. In the case of isopropyl ether and water the solubility of isopropyl ether in water was taken as 8 cc. in 100 cc. and of water in isopropyl ether as 2 cc. in 100 cc. at 25°+ C. These values are only approximately accurate, but are sufficiently close to the true values as not to make any appreciable difference.

The alkaloids used in the determinations were Merck and Company products. The isopropyl ether was obtained from the Carbide and Carbon Chemicals Corporation and conformed to the following specifications,

Boiling range	90 per cent distilled between 66° and 69° C. at 760-mm. pressure
Initial boiling point	Not less than 60° at 760-mm. pressure
Dry point	Not more than 70° at 760-mm. pressure
Color	Not more than 2% yellow
Specific gravity	0.723 to 0.729 at 20/20° C.
Residue	Not more than 0.1 per cent
Acidity	A 50-cc. sample does not contain more than the equivalent of 0.1 cc. normal acid or alkali
Suspended matter	Practically free from suspended matter

The product was subjected to careful distillation before use in order to get rid of the non-volatile residue. Only that portion distilling between 66°+ C. and 69°+ C. was used.

The methylene chloride used was obtained from the Advance Solvents and Chemical Corporation and E. I. duPont de Nemours and Company. The two products were essentially the same and conformed to the following specifications:

Color	Water white
Odor	No foreign odor
Boiling range	39.2° C. to 40° C. at 760 mm.
Specific gravity	1.33 at 15°/4° C.
Residue	None
Moisture	No cloud at −24° C.
Acidity	Less than 0.001 per cent calculated as HCl

These products were also subjected to careful distillation in order to get rid of any non-volatile residue.

The experiments were carried out in narrow glass-stoppered bottles in a constant temperature bath regulated at 25° C. to ± 0.1° The bottles were so arranged in the bath that they could be turned end over end at a rate of about 20 revolutions per minute. At the end of one hour the bottles were allowed to stand in the bath until the two layers were completely separated and clear, after which time 25 cc. of the non-aqueous solvent was pipetted off, the solvent allowed to evaporate spontaneously and the residue dried to constant weight in a desiccator over sulfuric acid. The alkaloids were determined gravimetrically.

The technique used is essentially the same as that used by Marden and Elliott (2), only differing in minor details, as to time, etc.

EXPERIMENTAL.

Strychnine.—The distribution coefficients of strychnine in the systems (*a*) water and isopropyl ether (*b*) water and a 3:1 mixture of isopropyl ether-methylene chloride, and (*c*) water and methylene chloride were determined. For these determinations 100 cc. of aqueous alkaloidal solution, 40 cc. of immiscible solvent and 5 cc. of ammonia water were used in each case. The results obtained are given in Table V.

TABLE V.—DISTRIBUTION RATIO OF STRYCHNINE BETWEEN WATER AND ISOPROPYL ETHER.

Wt. Strychnine in Sample.	Wt. Found in 25 Cc. Isopropyl Ether.	Wt. in Isopropyl Ether Layer 32 cc.	Wt. in Aqueous Layer 113 cc.	Concn. (d) in 10 Cc. H_2O / Conc. in 10 Cc. Non-Aqueous Solvent.
0.0103	0.0027	0.0035	0.0068	0.599
0.0123	0.0032	0.0041	0.0082	0.608
0.0166	0.0044	0.0056	0.0110	0.594
			Average	0.600

As may be seen from Table V, the value of (d) is 0.600. This means that 0.6 of the strychnine remains in the aqueous layer after one extraction, and upon extracting 50 cc. of the aqueous solution with 10-cc. portions of isopropyl ether (using 14 cc. for the first portion to allow for saturation of the aqueous layer) the value of (d) would be

$$\frac{0.6 \times 50}{0.6 \times 50 + 10} = \frac{30}{40} = 0.75$$

and it may be calculated that twelve such extractions $\left(\frac{1}{1.3}\right)^{12}$, would not remove much over 96.5 per cent of the alkaloid from aqueous solution, indicating that isopropyl ether is entirely unsatisfactory for this purpose. Calculations:

$$\left(\frac{1}{1.3}\right)^{12} = 30.00; \quad \frac{1 \times 100}{30} = 3.33\%; \quad 100.00 - 3.33 = 96.67\%$$

TABLE VI.—DISTRIBUTION RATIO OF STRYCHNINE BETWEEN WATER AND A 3:1 MIXTURE OF ISOPROPYL ETHER-METHYLENE CHLORIDE.

Wt. Strychnine in Sample.	Wt. Found in 25 Cc. Isopropyl Ether-Methylene Chloride, Layer.	Wt. in Isopropyl Ether Methylene Chloride (Layer 32 Cc.).	Wt. in Water (Layer 113 Cc.).	(d.)
0.0381	0.0214	0.0274	0.0107	0.110
0.0481	0.0231	0.0296	0.0111	0.107
0.0546	0.0263	0.0336	0.0125	0.106
			Average	0.107

It will be observed from Table VI (d = 0.107) that about one-tenth of the strychnine remains in the aqueous solution when equal volumes of the two solvents are used, and therefore, if 50 cc. of the aqueous solution of the alkaloid is extracted with 10-cc. portions of a 3:1 mixture of isopropyl ether-methylene chloride (14 cc. for the first portion to allow for saturation of the aqueous layer) the value of (d) becomes

$$\frac{0.107 \times 50}{0.107 \times 50 + 10} = \frac{5.35}{15.35} = 0.384$$

and it would require only four extractions $\left(\frac{1}{2.8}\right)^{4}$ to remove over 99 per cent of the strychnine and six extractions to almost completely remove the alkaloid from aqueous solution.

When it is remembered that the specific gravity of the immiscible solvent is an important factor in alkaloidal assay procedures, especially in extracting the alkaloid from aqueous solution, and that the specific gravity of a 3:1 mixture of isopropyl ether-methylene chloride is less than water, such a mixture should prove satisfactory in the assay of nux vomica.

TABLE VII.—DISTRIBUTION RATIO OF STRYCHNINE BETWEEN WATER AND METHYLENE CHLORIDE.

Wt. Strychnine.	Wt. Found in 25 Cc. Methylene Chloride.	Wt. in Methylene Chloride Layer (40 Cc.).	Wt. in Aqueous Layer (105 Cc.).	(*d*.)
0.0811	0.0493	0.0788	0.0023	0.010
0.1204	0.0733	0.1168	0.0036	0.011
			Average	0.0105

As may be seen in the case of water and methylene chloride the distribution ratio is 0.0105. Thus, when equal mixtures of these liquids are used a large per cent of the strychnine passes into the methylene chloride layer and when 50 cc. of the aqueous solution is extracted with 10-cc. portions of methylene chloride the value of (*d*) becomes

$$\frac{0.010 \times 50}{0.010 \times 50 + 10} = \frac{0.5}{10.5} = 0.048$$

and two extractions with 10-cc. portions $\left(\frac{1}{21}\right)^2$ will remove over 99.5 per cent of the strychnine from aqueous solution. This indicates very clearly that methylene chloride is satisfactory for this purpose.

To prove the conclusion that two 10-cc. portions of methylene chloride will extract practically all of the strychnine from aqueous solution, samples were prepared and extracted, using 1 cc. of ammonia water. The results are given in Table VIII.

TABLE VIII.

Wt. Strychnine.	Total Weight Found.	Percentage Found.
0.1000	0.0994	99.4
0.1000	0.0995	99.5
	Average	99.45

The extractions were carried out by uniformly shaking the separatory funnels for two minutes in each extraction and then allowing sufficient time for the separation of the two layers.

Brucine.—The amount of brucine extracted from aqueous solution with isopropyl ether is so small that it is difficult to obtain the correct distribution ratio. Check results were not obtained; however, it is clear from the results obtained that isopropyl ether would not be satisfactory for this purpose.

A 3:1 mixture of isopropyl ether-methylene chloride will extract more brucine from aqueous solution than isopropyl ether alone, but in this case also, the number of extractions required are too great for the solvent to have any practical value.

The distribution ratio of brucine between water and methylene chloride was found to be 0.098. This would indicate that brucine could be removed from aqueous solution by relatively few extractions using methylene chloride; however, more extractions would be required for brucine than for strychnine.

Atropine.—The distribution coefficients of atropine in the systems (*a*) water and isopropyl ether, (*b*) water and a 3:1 mixture of isopropyl ether and methylene chloride, and (*c*) water and methylene chloride are given in Tables IX, X and XI.

TABLE IX.—USING ISOPROPYL ETHER.

Wt. Atropine.	Wt. in 25 Cc. Isopropyl Ether.	Wt. in Isopropyl Ether Layer (32 Cc.).	Wt. in Aqueous Layer (113 Cc.).	(*d*.)
0.0284	0.0051	0.0062	0.0219	0.943
0.0401	0.0073	0.0093	0.0307	0.935
			Average	0.939

Using equal volumes of the solvent and water about as much atropine remains in the aqueous solution after one extraction as is extracted. Obviously, it would require too many extractions with this solvent to be of practical value.

TABLE X.—USING A 3:1 MIXTURE OF ISOPROPYL ETHER-METHYLENE CHLORIDE.

Wt. Atropine.	Wt. in 25 Cc. Immiscible Solvent.	Wt. in Immiscible Solvent Layer (32 Cc.).	Wt. in Aqueous Layer (133 Cc.).	(*d*.)
0.0821	0.0301	0.0409	0.0412	0.308
0.1004	0.0366	0.0498	0.0506	0.301
0.1164	0.0432	0.0587	0.0577	0.301
				Average 0.306

When a 3:1 mixture of isopropyl ether and methylene chloride is used the value of (*d*) is 0.306. It would require 10 extractions with 10-cc. portions of such a mixture to extract 96.5 per cent of the atropine from 50 cc. of aqueous solution. It is clear, therefore, that such a mixture would not be satisfactory for this purpose.

TABLE XI.—USING METHYLENE CHLORIDE.

Wt. Atropine.	Wt. in 25 Cc. Methylene Chloride.	Wt. in Methylene Chloride Layer (40 Cc.).	Wt. in Aqueous Layer (105 Cc.).	(*d*.)
0.0804	0.0397	0.0635	0.0169	0.101
0.1005	0.0484	0.0774	0.0220	0.109
0.1205	0.0584	0.0934	0.0261	0.107
				Average 0.106

As may be seen from Table XI ($d = 0.106$), methylene chloride is a much better immiscible solvent to extract atropine from aqueous solution than a 3:1 mixture of isopropyl ether-methylene chloride. Calculations show that five 10-cc. portions of methylene chloride will remove 99.5 per cent from aqueous solution, which is about the same amount as may be removed by five 10-cc. portions of chloroform. Thus, methylene chloride might be used as a satisfactory substitute for chloroform in extracting atropine from aqueous solution.

Quinine.—The distribution ratio of quinine in the systems (*a*) water and isopropyl ether, (*b*) water and methylene chloride and (*c*) water and a 3:1 mixture of isopropyl ether-methylene chloride is small in each case. Tables XII, XIII and XIV show the results obtained in the above cases.

TABLE XII.—DISTRIBUTION RATIO OF QUININE BETWEEN WATER AND ISOPROPYL ETHER.

Wt. Quinine.	Wt. in 25 Cc. Isopropyl Ether.	Wt. in Isopropyl Ether Layer (32 Cc.).	Wt. in Aqueous Layer (113 Cc.).	(*d*.)
0.0662	0.0390	0.0624	0.0038	0.024
0.1036	0.0609	0.0974	0.0062	0.026
				Average 0.025

Using the value ($d = 0.025$) calculations show that three washings with isopropyl ether would remove over 99.9 per cent of quinine from aqueous solution.

TABLE XIII.—DISTRIBUTION RATIO OF QUININE BETWEEN WATER AND A 3:1 MIXTURE OF ISOPROPYL ETHER-METHYLENE CHLORIDE.

Wt. Quinine.	Wt. in 25 Cc. Immiscible Solvent.	Wt. in Immiscible Solvent Layer (32 Cc.).	Wt. in Aqueous Layer (113 Cc.).	(*d*.)
0.0801	0.0462	0.0739	0.0062	0.033
0.0951	0.0534	0.0854	0.0097	0.043
0.1002	0.0585	0.0936	0.0066	0.031
				Average 0.035

A 3:1 mixture of isopropyl ether-methylene chloride would be satisfactory for extracting quinine from aqueous solution; however, based on the distribution ratio (d = 0.035) it is not quite as good as isopropyl ether alone.

TABLE XIV.—DISTRIBUTION RATIO OF QUININE BETWEEN WATER AND METHYLENE CHLORIDE.

Wt. Quinine.	Wt. in 25 Cc. Methylene Chloride.	Wt. in Methylene Chloride Layer (40 Cc.).	Wt. in Aqueous Layer (105 Cc.).	(d.)
0.1014	0.0628	0.1004	0.0010	0.0039
0.1200	0.0743	0.1188	0.0012	0.0041
				Average 0.004

Thus, it may be seen that methylene chloride is exceedingly efficient as an immiscible solvent for extracting quinine from aqueous solution. Two 10-cc. portions will almost completely extract the quinine from 50 cc. of aqueous solution. Where the value of (d) is so small as in the above case the solubility of methylene chloride in water and water in methylene chloride should be taken into consideration. These values could not be found in the literature, and therefore were not considered in calculating the distribution ratios.

Caffeine.—The distribution ratios of caffeine in the systems (a) water and isopropyl ether (b) water and methylene chloride and (c) water and a 3:1 mixture of isopropyl ether-methylene chloride indicate that any of the three solvents could be used for extracting caffeine from aqueous solution. For some reason good results could not be obtained; however, sufficiently accurate data were obtained to justify the conclusion that a 3:1 mixture of isopropyl ether-methylene chloride or methylene chloride alone would be highly satisfactory for this purpose.

From a consideration of the physical properties, isopropyl ether and methylene chloride appear to be well suited for use in alkaloidal assay work. Isopropyl ether has a specific gravity of 0.723–0.729 at 20° C., and a boiling point of about 67° C. Methylene chloride has a specific gravity of 1.33 at 15° C. and a boiling point of about 40° C. By using a 3:1 mixture of isopropyl ether and methylene chloride, therefore, a solvent is obtained which has a specific gravity less than water. Such a condition is desirable when one wishes to extract an alkaloid from the immiscible solvent by use of acidulated water. On the other hand, if it is desired that the alkaloid be removed from aqueous solution methylene chloride alone is to be preferred, because it will constitute the bottom layer and thus may be drawn off from the aqueous layer in the separatory funnel.

CONCLUSIONS.

1. The solubilities of atropine, caffeine, quinine and strychnine in isopropyl ether, methylene chloride and mixtures of these solvents have been determined.

2. From a consideration of these solubilities, isopropyl ether and methylene chloride should prove to be valuable solvents in the quantitative estimation of alkaloids.

3. The distribution coefficients of atropine quinine and strychnine in the systems (a) water and isopropyl ether, (b) water and methylene chloride, and (c) water and a 3:1 mixture of isopropyl ether-methylene chloride have been determined.

4. The conclusion is reached from a study of the distribution coefficients that these solvents possess definite possibilities as immiscible solvents to be used in extracting certain alkaloids from aqueous solution.

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STUDIES ON CALCIUM CREOSOTATE. I. CHEMICAL OBSERVATIONS ON THE WATER-SOLUBLE CONSTITUENTS.*[1]

BY EDWIN J. FELLOWS.

According to Gordonoff (1) creosote is more extensively used than any other therapeutic agent in the treatment of pulmonary disorders. The oral administration of creosote is attended with numerous objections the most pronounced of which, even after small doses, is its tendency to produce nausea and vomiting. Various attempts have been made to overcome these objectionable features of creosote therapy by diverse modifications of creosote and its constituents.

Calcium creosotate, prepared by the interaction of equal parts of calcium oxide and creosote, is a dark brown powder of empyreumatic odor and phenolic taste, which has enjoyed extensive clinical use as a creosote substitute for a period of more than twenty years. In view of the fact that a search of the literature discloses an almost complete lack of information on this drug and particularly since it has been admitted to U. S. P. XI, it would seem advisable to subject it to a thorough investigation.

The U. S. P. states that calcium creosotate is a mixture of the calcium compounds of creosote. When equal parts of calcium oxide and creosote are allowed to interact, the mixture attains a rather high temperature. This would lead one to suspect that the original creosote might be somewhat altered in the course of preparation of calcium creosotate. It would therefore seem important to determine whether or not calcium creosotate approximates creosote chemically.

All attempts to extract calcium creosotate with organic solvents were unsuccessful. When extracted with water a small percentage of the calcium creosotate goes into solution; the major portion of the drug, however, is water insoluble. Since aqueous solutions of calcium creosotate are to be used in certain future experiments the present investigation has been limited to a study of the chemical nature of the water-soluble constituents of the drug. An aqueous solution of calcium creosotate which would contain a definite amount of the powdered drug would be desirable. Since this is impossible solubility experiments were carried out in an attempt to establish a relationship between the amount of water-soluble constituents and

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