

were also corroborated by roentgenological studies made by the authors on rats with the kind assistance of Dr. M. Ostro, externe in roentgenology, Johns Hopkins Hospital, and roentgenologist, Sinai Hospital, Baltimore. In view of these gratifying results, it is deemed worth while to publish a description of the methods of experimentation for the benefit of those interested in this line of work. While the procedures employed may not be ideal, they were found to be quite adequate, and further investigation along these lines may lead to greater improvements of technique in a domain of pharmacology which has not yet been satisfactorily developed.

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A STATISTICAL STUDY OF THE PHARMACOPŒIAL CONSTANTS OF OLEUM CHENOPODII.*

BY JAMES C. MUNCH AND WILLIAM F. REINDOLLAR.

During the five-year period from 1925 to 1929, inclusive, authentic samples of normal and high-test wormseed oil were collected at the stills. The ascaridol content, specific gravity at $\frac{25^{\circ}}{25^{\circ}}$ C., specific rotation, refractive index at 20° C. and solubility in 70% alcohol were determined by the methods of the tenth Pharmacopœia (6). Pertinent data upon thirty-nine samples of normal and seven samples of high-test oil have been subjected to statistical analysis. Because of the difficulty of obtaining authentic oils a larger number of results could not be obtained. A detailed study has been made of the normal oils, but because of limited data less attention has been paid to high-test oils.

The values for ascaridol and the physical constants of the normal oils are given in Table I. The sums of the individual values of each determination have been divided by the number of samples to obtain the "mean." The deviation of each individual value from the mean has been squared. The sum of these squares has been divided by the number of observations to obtain the average of the squares. The square root of this average value is termed the "Standard Deviation." The standard deviation is a statistical measure of the accuracy of a series of determinations, and is expressed in the same order of magnitude as the mean. In order to obtain figures by which comparisons of the accuracy of different determinations may be made the "Coefficient of Variation" is calculated. This is obtained by dividing the standard deviation by the mean and multiplying the quotient by one hundred. In other words it is that per cent of the mean which is represented by the standard deviation. A series of results which differs but slightly from the mean, such as those of specific gravity or refractive index, show small values for

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the standard deviation and coefficient of variation. In Table I the coefficient of variation for these determinations was less than one, which means that the standard deviation was less than one per cent of the mean. Conversely a series of determinations presenting greater departures from the mean will have a larger standard deviation, as reflected by a larger coefficient of variation.

The "Probable Error" (*PE*) is a statistical measure of accuracy. In general it is two-thirds of the standard deviation. It may be determined by adding all of the deviations from the mean arithmetically (without regard to sign); dividing this sum by the number of observations gives the average deviation and the product of this by the constant 0.8453 gives the *PE*.

The "Correlation Coefficient" (*r*) measures the degree of co-relationship or the proportionate change in one variable of a pair when the other undergoes successive changes in magnitude. It is an abstract number which ranges from 1.00 (perfect agreement) to 0.00 (no relationship). If one value tends to increase with increasing values of the other, correlation is said to be direct and the sign is positive. If one value decreases as the other increases, correlation is inverse and the sign is negative. Higher degrees of correlation give values more closely approaching unity. However, the correlation coefficient is not a direct measure of the degree of correlation. For instance when $r = 0.90$ the degree of correlation is three times as certain as when $r = 0.60$, and eleven times as certain as when $r = 0.30$. It must be remembered that *r* is a measure of linear correlation. When relationship is not linear it gives spurious values and other measures of relationship must be used. In order to be significant the correlation coefficient must be greater than three times its probable error. The formula for calculating *r* is:

$$r = \frac{\Sigma XY}{N\sigma_X\sigma_Y}$$

in which *X* and *Y* represent the deviations of each pair of values from their respective means, Σ represents the summation of these values, *N* the number of pairs of observations, and σ_X and σ_Y are the standard deviations of *X* and *Y*, respectively.

The formula for the Probable Error of *r* is

$$PE_r = 0.6745 \frac{1 - r^2}{\sqrt{N}}$$

Before computing the correlation coefficient a scatter diagram should be constructed. The more accurate series of values, or those in which we have the most confidence, are taken as abscissae, the other series as ordinates. After *r* has been determined a regression line is fitted to the data by the formula

$$(Y - Y') = r \frac{\sigma_Y}{\sigma_X} (X - X')$$

The value for *r* is 0.89, its probable error = 0.02 and the equation of the regression line is

$$Y = 0.00092 X + 0.9076.$$

The correlation coefficient between ascaridol and the physical constants have been consolidated in Table II as well as the correlation between the constants.

TABLE I.—PHARMACOPŒIAL CONSTANTS OF NORMAL OIL OF CHENOPODIUM 1926-1929, INCLUSIVE.

Number.	Ascaridol.	Sp. gr. av. at $\frac{25^{\circ}}{26^{\circ}}$.	Sp. rotat. at 25°.	Ref. index at 20°.	Volumes of 70% alcohol for complete solution.
1	64	0.9669	-6.5	1.4751	7
2	67	0.9654	-6.4	1.4749	7
3	63	0.9645	-6.5	1.4748	7
4	66	0.9685	-6.0	1.4749	6
5	72	0.9701	-5.1	1.4752	5
6	78	0.9781	-4.7	1.4749	3
7	71	0.9725	-7.5	1.4736	2
8	75	0.9759	-7.1	1.4735	2
9	64	0.9640	-6.9	1.4732	4
10	74	0.9751	-5.9	1.4738	3
11	71	0.9719	-6.9	1.4738	3
12	77	0.9796	-5.0	1.4741	3
13	65	0.9684	-6.6	1.4739	2
14	63	0.9618	-8.7	1.4740	3
15	79	0.9799	-5.9	1.4737	2
16	66	0.9664	-7.6	1.4734	3
17	58	0.9641	-5.0	1.4750	7
18	55	0.9580	-6.5	1.4749	8
19	63	0.9667	-5.4	1.4745	6
20	63	0.9651	-5.5	1.4750	7
21	70	0.9776	-4.7	1.4746	3
22	68	0.9728	-5.0	1.4746	3
23	61	0.9665	-5.6	1.4752	3
24	74	0.9759	-5.3	1.4747	2
25	71	0.9725	-5.4	1.4745	2
26	63	0.9617	-6.8	1.4749	3
27	76	0.9792	-5.0	1.4745	2
28	67	0.9746	-4.6	1.4747	2
29	66	0.9724	-4.8	1.4745	2
30	60	0.9616	-6.7	1.4752	5
31	58	0.9593	-6.4	1.4750	6
32	50	0.9549	-6.4	1.4754	9
33	60	0.9622	-7.6	1.4749	5
34	62	0.9715	-5.8	1.4747	3
35	67	0.9629	-7.6	1.4753	4
36	56	0.9510	-8.2	1.4752	8
37	64	0.9748	-5.5	1.4744	3
38	73	0.9749	-4.4	1.4750	3
39	76	0.9765	-5.9	1.4748	2
Sum	2596	37.7857	-237.4	57.5083	160
Mean	66.6	0.9689	-6.1	1.4746	4.1
Stand. dev.	6.86	0.0071	1.06	0.00058	2.06
C. V.	10.5	0.73	17.4	0.04	50

Partial correlation coefficients have been calculated between two variables when one, two or three other variables are fixed. The formula for the partial correlation coefficient is

$$r_{ab,c} = \frac{r_{ab} - (r_{ac})(r_{bc})}{\sqrt{(1 - r_{ac}^2)(1 - r_{bc}^2)'}}$$

in which the various subscripts represent the pairs of variables compared. After the partial correlation coefficients have been calculated in which one variable is fixed, an extension of this formula is used for the fixation of two or more variables.

TABLE II.—CORRELATION COEFFICIENTS FOR ASSAYS OF OIL OF CHENOPODIUM.

1.		5.	
	<i>P.E.</i>		<i>P.E.</i>
Ascaridol/SpGr.....	0.89±0.02	SpGr/Rot.....	-0.60±0.07
Asc:Gr/Rot.....	0.89		
Asc:Gr/nD.....	0.87		
Asc:Gr/Alc.....	0.76	6.	
Asc:Gr/Rot, nD.....	0.85	SpGr/nD.....	-0.42±0.09
Asc:Gr/Rot, Alc.....	0.80	SpGr:nD/Rot.....	-0.69
Asc:Gr/nD, Alc.....	0.77		
Asc:Gr/Rot, nD, Alc.....	0.79	7.	
		SpGr/Alc.....	-0.74±0.05
2.		SpGr:Alc/Asc.....	-0.29
Ascaridol/Rot.....	-0.38±0.09	SpGr:Alc/Rot.....	-0.77
Asc:Rot/Gr.....	+0.42	SpGr:Alc/nD.....	-0.67
Asc:Rot/nD.....	-0.59	SpGr:Alc/Rot, nD.....	-0.61
Asc:Rot/Alc.....	-0.33		
		8.	
3.		Rot/nD.....	-0.21±0.11
Ascaridol/nD.....	-0.40±0.09		
Asc:nD/Gr.....	-0.06	9.	
Asc:nD/Rot.....	-0.53	Rot/Alc.....	+0.23±0.11
Asc:nD/Alc.....	+0.02		
		10.	
4.		nD/Alc.....	0.56±0.07
Ascaridol/Alc.....	-0.73±0.05	nD:Alc/Rot.....	0.61
Asc:Alc/Gr.....	-0.23		
Asc:Alc/Rot.....	-0.66		
Asc:Alc/nD.....	-0.66		
Asc:Alc/Rot, nD.....	-0.50		

Asc represents ascaridol.

SpGr or Gr represents specific gravity.

nD represents index of refraction.

Alc represents alcohol.

Rot represents rotation.

The correlation coefficient between ascaridol and specific gravity was found to be 0.89. When the rotation or the refractive index was fixed no significant change in r was produced. When, however, the alcohol solubility was fixed the value decreased to 0.76. This is interpreted to mean that the alcohol solubility influenced the ascaridol: specific gravity correlation. Since the correlation coefficient of ascaridol to alcohol solubility was -0.73 and the correlation of specific gravity to alcohol solubility was -0.74 , it is not possible to determine which interrelationship is responsible for the decrease in the partial correlation coefficient. Fixation of the rotation and the index of refraction, as well as the alcohol solubility, failed to cause any further change. This is interpreted as further confirmation of the inter-relationship of ascaridol and specific gravity but not of the other variables.

A number of other partial correlation coefficients were calculated without revealing more significant relationships. It is interesting to note that there is a

significant inverse relationship between ascaridol and alcohol solubility ($r = -0.73 \pm 0.05$).

In studies on high-test oil the correlation coefficient for ascaridol to specific gravity was 0.85 ± 0.07 . Because of insufficient data further correlation coefficients were not determined.

Calculations of the correlation coefficients and partial correlation coefficients have been greatly simplified by the use of approximation formulas and tables developed in the course of this type of statistical research (1, 2, 3, 4, 5, 6, 7, 8).

CONCLUSIONS.

- (a) Ascaridol shows a significant direct relationship to specific gravity ($r = 0.89 \pm 0.02$) and inverse relationship to alcohol solubility ($r = -0.73 \pm 0.05$).
- (b) These relationships are confirmed by partial correlation coefficients.
- (c) The other variables show no significant relationship.

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HYDROLYSIS OF ACETYLSALICYLIC ACID IN ETHANOL, GLYCEROL AND ETHANOL-GLYCEROL SOLUTIONS.*

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INTRODUCTION.

Conversion of the acetic acid ester of salicylic acid into its components in aqueous solution by the hydrolytic action of the solvent occurs with comparative rapidity. In the effort to prevent this disintegration when it is desired to dispense the analgesic in solution form it has been suggested (1) that the compound be dissolved in a menstruum consisting of portions of ethyl alcohol and glycerol. It is evident, however, since the U. S. P. grade of the latter rarely contains more than 95 per cent of the tri-hydric alcohol, while ethanol of the same quality contains a similar proportion of water—that hydrolysis cannot be entirely suppressed by the employment of these vehicles. It seemed desirable to learn to what extent the phenomenon would be induced by varying the water content of ethanol and glycerol

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