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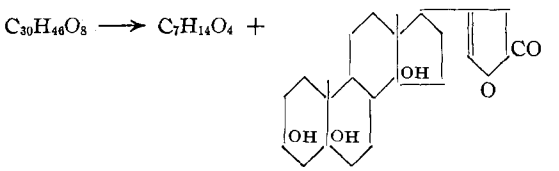
THE POTENCY OF PERIPLOCYMARIN, BUFOTALIN AND DESACETYL-OLEANDRIN.*

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):

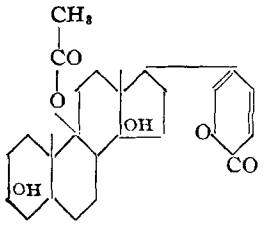


Periplocymarin Cymarose Periplogenin

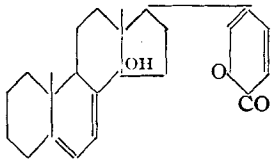
In 1913 Wieland and Weil (5) succeeded in crystallizing a cardiac principle, bufotalin, from the skin of the common European toad, *Bufo vulgaris* (*Bufo bufo bufo*), a substance resembling Abel's bufagin (6) but not identical with it. The constitution of bufotalin was repeatedly illucidated by Wieland (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.

[1] From the Lilly Research Laboratories, Indianapolis, Indiana.



Bufotalin



Scillaridin A

similar to that of scillaridin A (12). We attempted to isolate bufotalin from the secretion of *B. vulgaris*, but failed (13), while Wieland was always successful with one exception (14). It is not possible to account for the discrepancy at present.

Windaus and Westphal (15) in 1925 investigated the chemistry of oleandrin, a cardiac glycoside of the leaves of the plant *Nerium oleander*. Recently a similar product named folinerin has been studied by Flury and Neumann (16), and its potency has been determined in our laboratory (17). Additional chemical work carried out by Neumann (18) and Tschesche (19) shows conclusively that oleandrin is a derivative of gitoxigenin with an acetyl group on C_{16}, coupled with a molecule of oleandrose, and that oleandrin and folinerin are identical. The word folinerin can therefore be dropped from the scientific literature. The acetyl-free oleandrin which also occurs in nature is called desacetyl-oleandrin.

Although our chemical knowledge of the above substances has advanced, the exact potency of periplocymarin, bufotalin and desacetyl-oleandrin has never been determined. It is the purpose of this paper to report the data obtained with the three compounds. The bufotalin used in our experiments was courteously supplied by Professor Heinrich Wieland, München, Germany. Desacetyl-oleandrin was secured from Dr. Wilhelm Neumann, Würzburg, Germany, through the kindness of Dr. Rudolf Tschesche, Berlin. Periplocymarin was prepared from *Periploca græca*, crystallizing with one molecule of methyl alcohol, sintering at 138° C., and beginning to melt at 148° C. (corrected).

Our experimental procedures were the same as published previously (20). To effect a 0.1 per cent stock solution, periplocymarin was dissolved in 28.5 per cent ethyl alcohol (by volume), bufotalin in 47.5 per cent and desacetyl-oleandrin in 57 per cent. The results are shown in Tables I, II and III.

TABLE I.—AVERAGE FATAL DOSE OF PERIPLOCYMARIN, BUFOTALIN AND DESACETYL-OLEANDRIN IN CATS.

Drug.	Cat Number.	Sex.	Body Weight, Kg.	Fatal Dose, Mg. per Kg.	Average Fatal Dose ± Probable Error, Mg. per Kg.
Periplocymarin	1675	M	2.447	0.148	
	1676	F	2.116	0.139	
	1678	F	2.637	0.126	
	1679	F	2.196	0.174	
	1681	M	2.855	0.151	0.151 ± 0.004
	1682	M	2.417	0.160	
	1683	F	1.694	0.125	
	1684	F	2.158	0.145	
	1685	F	2.683	0.186	
	1686	F	2.182	0.157	

Bufotalin	1899	M	2.592	0.103	
	1900	M	2.547	0.116	
	1901	F	2.394	0.132	
	1902	M	2.264	0.134	
	1903	F	1.968	0.146	
	1904	F	2.760	0.101	0.130 ± 0.006
	1905	F	2.182	0.152	
	1906	M	2.151	0.160	
	1907	F	2.068	0.168	
	1908	F	1.720	0.083	
Desacetyl-oleandrin	1959	F	2.052	0.320	
	1960	F	2.227	0.258	
	1961	F	2.083	0.358	
	1962	F	2.334	0.230	
	1963	M	2.262	0.287	
	1964	F	1.949	0.409	0.308 ± 0.016
	1965	F	1.855	0.300	
	1966	F	2.242	0.252	
	1967	F	1.918	0.448	
	1968	F	1.854	0.219	

TABLE II.—MINIMAL SYSTOLIC DOSE OF PERIPLOCYMARIN, BUFOTALIN AND DESACETYL-OLEANDRIN IN FROGS.

Drug.	Concentration.	Dose, Mg. per Gm.	Number in Systole/ Number of Frogs Used.	Minimal Systolic Dose, Mg. per Gm.
Periplocymarin	1:20,000	0.00054	0/4	0.00318
		0.00064	0/4	
		0.00073	0/4	
		0.00909	0/4	
	1:2,000	0.00182	0/4	
		0.00273	3/8	
		0.00318	3/4	
		0.00364	7/8	
	1:1,000	0.00455	3/4	
		0.00909	4/4	
Bufotalin	1:500	0.00750	0/4	0.00917
		0.00833	2/8	
		0.00917	3/4	
Desacetyl-oleandrin	1:1,000	0.00208	0/2	0.01167
		0.00416	0/2	
		0.00667	0/2	
		0.00833	0/4	
		0.01000	0/4	
		0.01083	1/4	
		0.01167	3/4	
		0.01250	2/2	

It should be noted that in cats bufotalin proves to be less potent than areno-, quercico-, gama- and virido-bufagins, the results of which were recorded in a previous report (21); but it is decidedly more active than regularo-, vallicepo-, fowlero-, cino- and marino-bufagins. It is also more powerful than vulgaro-

bufotoxin, the conjugation product of bufotalin with suberyl-arginine isolated from the secretion of *Bufo vulgaris* (*Bufo bufo bufo*) (8), (13). The ratio of activity, between bufotalin and its bufotoxin is approximately 1:2.3.

TABLE III.—MINIMAL EMETIC DOSE AND PERSISTENCE OF ACTION OF PERIPLOCYMARIN, BUFOTALIN AND DESACETYL-OLEANDRIN IN CATS.

Drug.	Cat Number.	Sex.	Body Weight, Kg.	Initial Dose, Mg. per Kg.	Vomiting Occurred.	Final Fatal Dose, Mg. per Kg.	Interval between Initial and Final Fatal Doses, Hours.
Periplocymarin	1702	M	2.397	0.07	0	0.136	2.4
	1705	M	3.161	0.07	0	0.178	2.2
	1703	M	1.810	*0.08*	+	0.125	3.1
	1704	M	2.092	*0.08*	+	0.147	20.5
Bufotalin	1919	F	2.306	0.05	0	*	
	1920	F	1.755	0.05	+	0.173	1.9
	1921	F	2.333	0.05	0	*	
	1915	M	2.598	*0.06*	+	0.142	1.5
	1922	F	2.016	*0.06*	+	0.180	5.0
	1916	F	1.760	0.07	+	0.157	7.8
	1918	M	2.438	0.07	+	0.140	4.7
	1917	F	1.950	0.08	+	0.202	23.9
Desacetyl-oleandrin	1979	F	2.342	0.04	0	*	
	1976	M	2.157	0.05	0	*	
	1973	M	2.578	0.06	+	0.278	4.5
	1974	F	2.405	0.06	0	0.297	2.0
	1975	F	1.995	0.06	0	0.312	3.7
	1977	F	1.776	*0.07*	+	0.362	4.0
	1978	F	1.982	*0.07*	+	0.269	4.0
	1970	M	1.982	0.08	+	0.287	2.0
	1972	M	2.120	0.09	+	0.262	6.3
	1971	F	2.050	0.10	+	0.210	7.0

* Not determined.

Periplocymarin has practically the same cat unit (Table I) as scillaren A (20), regularo-bufagin (21) and coumingine hydrochloride (22). Its potency is lower than that of convallatoxin, β- and α-antiarins, ouabain, calotropin and cymarin, but it is more powerful than the remaining cardiac glycosides and alkaloids so far investigated in this laboratory.

In frogs (Table II), periplocymarin yields a larger minimal systolic dose than scillaren A and coumingine hydrochloride. Similarly bufotalin having the same cat unit as cymarin is 15.3 times less toxic than the latter to frogs. The disagreement of results by the cat method as compared with the frog method is a subject that will be discussed more fully at a later date.

The minimal emetic dose of periplocymarin (0.08 mg. per Kg.) in cats is equivalent to 53 per cent of the cat unit, and that of bufotalin (0.06) 46 per cent of its cat unit (Table III). Neither substance has a high persistence of action, for the final fatal doses of all the cats used for the emesis test are within the limits of the control series (see Table I).

Desacetyl-oleandrin, which is oleandrin minus the acetyl group, is definitely less potent than oleandrin (folinerin) either in cats or in frogs, particularly in the

latter (Tables I and II) (17). Neumann and Lindner (24) reported similar results. This is interesting, because in our previous work (23), (22) it was demonstrated that acetylation of cino-bufagin, marino-bufagin or cassaine reduced the cardiac activity in each case. Conversely, one would expect that the removal of the acetyl group increases the potency, but obviously with desacetyl-oleandrin this postulate breaks down. The minimal systolic dose of desacetyl-oleandrin in cats is 75 per cent larger than that of folinerin. In other words, the emetic action becomes greater when the acetyl group is intact. In the preceding article (22), it was shown that acetyl-cassaine is more emetic than cassaine, Gm. for Gm. With cino-bufagin, the acetyl derivative is equally effective in causing vomiting (23). Since acetyl-cino-bufagin has a larger molecule than the parent substance, the former is thus more emetic than the latter, molecule for molecule. On the other hand, acetyl-marino-bufagin is much less powerful in inducing emesis than marino-bufagin (22), just the reverse of the foregoing examples. This goes to show that it is difficult to predict the changes in physiological action following acetylation. It seems that different results may occur in the acetylation by nature as compared with that by laboratory methods. The deletion of acetyl group from oleandrin contributes another interesting feature; that is, oleandrin has a slow and persistent action (17), while desacetyl-oleandrin causes vomiting within 8 to 20 minutes (average 14 minutes), and also leaves the circulation more promptly. As shown in Table III, the final fatal doses with one exception (cat numbered 1971), determined 2 to 7 hours after the initial dose, all appear to lie within normal limits (compare Table I).

SUMMARY.

The potency of periplocymarin, bufotalin and desacetyl-oleandrin has been carefully determined.

Periplocymarin has approximately the same cat unit as scillaren A and coumingine hydrochloride. It is less potent than convallatoxin, β- and α-antiarins, ouabain, calotropin and cymarin.

Bufotalin is less powerful than areno-, quercico-, gama- and virido-bufagins, but more powerful than other bufagins so far investigated. It is 2.3 times more potent than vulgaro-bufotoxin, another cardiac principle occurring in the secretion of the same species of toads (*Bufo vulgaris* or *B. bufo bufo*).

Desacetyl-oleandrin is less active than the acetylated product oleandrin. Its effect appears to be more prompt and less persistent than that of oleandrin.

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THE DETECTION OF CARBON MONOXIDE IN MEDICINAL OXYGEN.*[1]

BY FREDERICK K. BELL AND JOHN C. KRANTZ,[2] JR.

INTRODUCTION.

The eleventh revision of the United States Pharmacopœia includes a general test for the detection of carbon monoxide in the medicinal gases, oxygen and carbon dioxide. The procedure employed is a modification of the method set forth by Teague (1). In the Teague method thoroughly scrubbed, dried air is allowed to pass over heated, highly purified iodine pentoxide from which it quantitatively liberates iodine. The entire apparatus must be swept for hours and sometimes for days with carbon monoxide-free air in order to obtain a negative blank. To make this method applicable to medicinal oxygen we (2 and 3) inserted an additional I_2O_5 tube through which the gas to be tested was previously washed to remove any carbon monoxide that might be present. Thus the gas to be tested could be employed to scrub the iodine pentoxide that was to be used in the test.

Since this method became official it has become the subject of careful investigation on the part of manufacturers of medicinal gases. The principal criticism of the method is the difficulty and time involved in the obtaining of a negative blank and the complete failure to obtain a negative blank in the hands of certain experimentors. Success or failure in this endeavor depends first on the quality of the iodine pentoxide employed and secondly on the careful observance of all experimental details set forth in the method.

The experiments set forth in this communication describe a method for the detection of carbon monoxide in oxygen without the use of the troublesome iodine pentoxide.

EXPERIMENTAL.

The method developed depends upon the reduction of palladious chloride to palladium by the carbon monoxide (4). The flocculent metallic palladium in suspension reacts with a solution of ammonium molybdate producing molybdenum blue, the intensity of which is propor-

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