[CONTRIBUTION FROM THE GEORGE WILLIAMS HOOPER FOUNDATION, UNIVERSITY OF CALIFORNIA, AND THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

Paralytic Shellfish Poison. II. Purification by Chromatography[1]

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Mussel poison concentrates prepared by ion exchange on Decalso[2] contain salts of other organic bases as well as inorganic salts. Sommer and Bendien[3] investigated the use of chromatography for further purification and found active carbon to be the most satisfactory adsorbent.

Preliminary tests were conducted to determine the optimum conditions for the chromatography. The results were accepted as the basis for all further work and corroborative tests have not been made using purer concentrates. Of the four carbons tested (Darco G-60, Norit A, and Nuchar C and XXX), Norit A appeared to be the most suitable. The mussel poison was not adsorbed completely from solutions much more dilute than 1.0 *N* in acid. Similarly, traces of ethanol in the extract caused a partial elution of the toxic material.

All of the succeeding runs were made by adsorbing the poison from 1.0 *N* hydrochloric acid solution. The mussel poison hydrochloride was retained by the carbon and the major portion of the inorganic as well as organic contaminants passed through at once. The use of distilled water to develop the chromatogram was sufficient to carry the poison through into the filtrate. The toxicity of the material in each fraction collected was calculated from the bioassay[4] and the determination of total solids. The most toxic fraction usually had a *p*H of about 2.3. The chromatography of a typical concentrate obtained by ion exchange on Decalso is described in Table I. Mussel poison hydrochloride with a toxicity greater than 3.3 MU./γ has been prepared by rechromatographing combined lots of material of high toxicity obtained from carbon columns. In this way the ash content of these samples has been reduced to 3–4%.

For crude mussel liver extracts with toxicities less than about 3.3 MU./mg., the concentration by ion exchange on Decalso proved unsatisfactory.[2] A method, which was suggested by the work of Sommer and Bendien,[3] was applied to this material with some success. This involved the direct chromatography of partially decolorized and defatted crude extracts on active carbon after removal of traces of ethanol by successive evaporations with water. It is possible to obtain fractions of mussel poison hydrochloride with toxicities greater than 1.0 MU./γ by this method (Table II). A decided advantage is that an over-all recovery of 60–80% is usually obtained. The poorer fractions may be rechromatographed for further purification. This method was not used for material of greater toxicity, since the Decalso procedure was less time-consuming and provided enriched poison concentrates in fairly good yield.

Since the preparation of the carbon for chromatography involved a rather lengthy procedure, it seemed advisable to attempt the repeated use

TABLE I

CHROMATOGRAM OF A TYPICAL DECALSO ELUATE[a]

Solvent	Volume, ml.	Poison recovered MU.	%	Total solids, g.	Toxicity, MU./γ
		Starting Material			
1 *N* HCl	100	612,000	100.0	5.1900	0.118
		Filtrates			
MeOH·HCl	20	<280		0.0008	
MeOH·HCl	15	<200		0.1615	
1 *N* HCl	50	<1,080		1.6454	
1 *N* HCl	25	<570		0.9078	
1 *N* HCl	25	810	0.1	.6434	0.0013
H_2O	25	1,670	0.3	.4211	.0040
H_2O	25	3,120	0.5	.2370	.0132
H_2O	25	43,200	7.1	.1432	.301
H_2O	40	238,000	38.9	.1659	1.44
H_2O	45	95,000	15.5	.0717	1.32
H_2O	50	18,800	3.1	.0457	0.410
H_2O	100	9,300	1.5	.0484	.192
H_2O	100	3,220	0.5	.0287	.112
MeOH·HCl	100	7,200	1.2	.2530	.0285
MeOH·HCl	100	<1,440		.0516	
		420,000	68.7	4.8252	

[a] The adsorbent was commercial acid-washed Norit A which was further digested with 10% hydrochloric acid. Treatment was repeated until a negative test for the ferric ion was obtained with 10% potassium ferrocyanide solution. The carbon was well washed with water and dried. A smooth suspension of 15 g. of the carbon in methanol containing 10 ml. of concentrated hydrochloric acid per liter was poured into a Pyrex column provided with a stopcock at the bottom and a cotton plug to support the carbon. The solvent was allowed to run through until level with the top of the adsorbent, and the amount remaining (38 ml.) was considered the hold-up of the carbon column (21 × 85 mm.). The column was operated under an air pressure of 125 cm. of water. The chromatogram was developed with distilled water. The final traces of material remaining on the column were eluted with the acidified methanol.

The use of dilute aqueous hydrochloric acid in place of acidified methanol in preparing the column for chromatography has little or no effect on the recovery of the poison. However, the rate of filtration of the water is more rapid in the latter type of column.

(1) The work described in this paper was done under a contract between the Chemical Corps, Camp Detrick, Frederick, Maryland, and the University of California and Northwestern University.

(2) H. Sommer, R. P. Monnier, B. Riegel, D. W. Stanger, J. D. Mold, D. M. Wikholm and E. S. Kiralis, THIS JOURNAL, **70**, 1015 (1948).

(3) W. M. Bendien and H. Sommer, *Proc. Soc. Exptl. Biol. Med.*, **48**, 715 (1941).

(4) The bioassay was carried out by the intraperitoneal injection of the poison into white mice. The mouse unit (MU) is the average lethal dose of poison that will kill a 20-g. mouse in fifteen minutes.

TABLE II

CHROMATOGRAPHIC FRACTIONATION OF CRUDE MUSSEL POISON EXTRACTS[a]

Solvent	Volume, ml.	Poison recovered MU.	%	Total solids, g.	Toxicity, MU./mg.
		Starting Material			
1 *N* HCl	500	410,000	100.0	20.0	20.5
		Filtrates			
MeOH·HCl	140				
1 *N* HCl	500	<11,400			
H_2O	248	2,820	0.7	0.166	1.70
H_2O	210	78,800	19.2	.546	144
H_2O	100	119,000	29.0	.125	952
H_2O	100	27,400	6.7	.096	284
H_2O	100	9,270	2.3	.087	107
H_2O	223	5,840	1.4	.120	48.9
H_2O	196	2,870	0.7		
H_2O	250	2,720	0.7		
H_2O	119	1,420	0.3		
MeOH·HCl	218	11,100	2.7		
		261,000	63.7		

[a] Acid-washed Norit A (60 g.) was used to prepare a 31 × 190 mm. column which had a hold-up of 138 ml.

of these columns. This provided some unexpected results. The used carbon gave a better return of eluted material as well as fractions of higher toxicity in most cases. However, after the passage of several lots through the same column or the use of very impure starting material, the column had to be discarded. A typical series of repeated runs with mussel poison prepared by ion exchange on Decalso is given in Table III. When crude material was chromatographed on a column of acid-washed Norit A which had been used once, a similar improvement in yield and enrichment was observed.

An experiment in which used carbon was reactivated by digestion with 10% hydrochloric acid indicated that it could be used again for chromatography of crude extracts with somewhat higher yields and greater enrichment than could be obtained with fresh acid-washed Norit A.

The adsorption behavior of mussel poison on acid-washed Norit A appears to be dependent upon the nature of the anion. Experiments in which solutions of mussel poison in 1.0 *N* hydrobromic, trichloroacetic and Reinecke acids were passed through columns of the active carbon provided widely varying results. The hydrobromide of the poison was found to be much more strongly adsorbed than the hydrochloride and was only eluted by acidified methanol. The trichloroacetate was not adsorbed under these conditions and passed through the column into the acid filtrate. The Reineckate was dissociated, the Reinecke acid being irreversibly adsorbed on the carbon and the poison reappearing as the hydrochloride in the eluate. None of these acids appeared

TABLE III

REPEATED CHROMATOGRAPHY OF MUSSEL POISON ON THE SAME COLUMN[a]

		Starting material[b] 1 *N* HCl	Hold-up MeOH·HCl	Acid filtrate 1 *N* HCl	Eluates: H_2O 1	2	3	4	5	6	MeOH·HCl	Total recov., %
1	Vol., ml.	25.0	8.0	25.0	11.0	9.0	12.5	15.0	20.0	25.0	25	
	*p*H				1.6	2.2	....	2.4	2.5	2.9		
	Poison, %	100.0	0.0	0.0	0.4	0.5	62.5	11.2		0.8	2.3	77.7
	Tox., MU./γ	0.0862			....	....	1.08	0.410		0.186		
2	Vol., ml.	25.0	7.0	25.0	10.0	10.0	10.0	10.0	10.0	30.0	25.0	
	*p*H				1.5	2.1	2.2	2.3	2.4	2.6		
	Poison, %	100.0	0.2	0.0	6.7	20.3	21.5	22.1	6.8	4.6	2.2	84.4
	Tox., MU./γ	0.0862			....	0.299	0.680		0.641	0.370		
3	Vol., ml.	25.0	7.0	25.0	10.0	10.0	10.0	11.5	25.0		25.0	
	*p*H				1.3	1.9	2.2	2.3	2.5			
	Poison, %	100.0	0.0	0.0	1.3	14.4	47.1	16.0	5.5		4.9	89.2
	Tox., MU./γ	0.0862			....	0.167	0.093	1.00	0.463			
4	Vol., ml.	25.0	9.0	23.0	11.0	10.0	11.5	12.0	25.0	25.0	50.0	
	*p*H				1.6	2.0	....	2.2	2.5	2.8		
	Poison, %	100.0	0.0	0.0	0.8	18.1	57.1	11.2	4.2		1.0	92.4
	Tox., MU./γ	0.437			....	0.268	0.926	0.451	0.225			
5	Vol., ml.	25.0	8.0	25.0	10.0	11.0	11.0	10.0	26.0	25.0	50.0	
	*p*H				1.2	....	....	2.5	2.7	2.9		
	Poison, %	100.0	0.0	0.8	15.6	28.8	28.7	15.3	4.5	0.4	1.5	95.6
	Tox., MU./γ	1.00			0.833	1.07	1.05	1.01	0.543	....		

[a] Three grams of acid-washed Norit A was used to prepare a 11.5 × 80 mm. column which had a hold-up of 9 ml. [b] The starting material was a Decalso eluate containing 152,000 MU. with a toxicity of 86.2 MU./mg. This material was dissolved in 75 ml. of 1.0 *N* hydrochloric acid and divided into three portions of 25 ml. each for the first three runs. The fourth run was carried out on combined fractions with toxicities greater than 0.296 MU./γ from the first three runs, a total of 42,900 MU. (0.436 MU./γ). The starting material for the fifth run was made up of fractions with toxicities greater than 0.908 MU./γ from the first four runs, a total of 88,200 MU. (1.00 MU./γ).

to offer any advantage over hydrochloric acid for chromatography.

The recommended procedure for purifying mussel poison concentrates by chromatographic fractionation on acid-washed Norit A is based on experience gained from carrying out over one hundred chromatograms. Though it is difficult to obtain exactly duplicate results even with aliquots of the same material on columns prepared in an identical manner, the average total recovery of poison in chromatography is from 60–80%. The most toxic fraction of the eluate contains 30–50% of the poison. This fraction will often show an enrichment of as great as twenty-fold if the starting material is a concentrate obtained by ion exchange on Decalso and as great as seventy-fivefold if the starting material is a crude, partially decolorized mussel poison extract. Poison concentrates obtained from ion exchange on Decalso contain 50–70% of the poison in the crude extract and usually have a toxicity of 0.07–0.14 MU./γ. Chromatography of these eluates on acid-washed Norit A results in a recovery of 30–50% of this poison with a toxicity greater than 1 MU./γ. The over-all recovery of poison with a toxicity greater than 1 MU./γ, after purification by ion exchange on Decalso and subsequent chromatography on acid-washed Norit A, is 18–30%.

Summary

1. A procedure is described for the chromatographic fractionation of mussel poison concentrates on active carbon.

2. Repeated use of carbon columns for the chromatographic fractionation of mussel poison hydrochloride usually results in a better recovery of poison as well as in fractions of higher toxicity.

3. The adsorption behavior of mussel poison on active carbon varies widely with different anions.

4. By chromatography on Norit A of mussel poison concentrates obtained from ion exchange on Decalso, it is possible to obtain a 30–50% yield of twenty-fold enriched material with a toxicity greater than 1 MU./γ.

5. The chromatography of partially decolorized, defatted, crude mussel poison extracts on Norit A yields 30–50% of seventy-five-fold enriched material.

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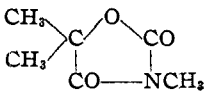
Some N-Alkyl-2,4-oxazolidinediones and their Anticonvulsant Properties

BY M. A. SPIELMAN AND GUY M. EVERETT

An earlier communication[1] has described the synthesis of some N-alkyl derivatives of 2,4-oxazolidinedione and their effect as analgesic agents. Subsequently, Everett and Richards[2] investigated the anticonvulsant properties of this type of compound, and as an outgrowth of their studies 3,5,5-trimethyl-2,4-oxazolidinedione under the trade name Tridione (I) has come into wide use in the treatment of petit mal epilepsy.[3] This paper presents results obtained in an expansion of the series.



I

The new N-alkyl-2,4-oxazolidinediones are much like those previously reported. They are liquids or low-melting solids, neutral in reaction and rapidly destroyed by strong aqueous alkali. They were synthesized along conventional lines as described below.

Experimental Part[4]

The parent 2,4-oxazolidinediones were prepared by the Stoughton method[5] in which an α-hydroxyester is condensed with urea by means of sodium ethoxide.

One α-hydroxyester is new. Methyl *n*-propyl ketone cyanhydrin on alcoholysis gave ethyl α-hydroxy-α-methylvalerate; b. p. 91–95° at 40 mm., $n^{25}D$ 1.4135. *Anal.* Calcd. for $C_8H_{16}O_3$: C, 59.98; H, 10.09. Found: C, 59.84; H, 9.88.

The following three new 2,4-oxazolidinediones were prepared by condensing the appropriate α-hydroxyester with urea.[5]

5-*n*-Propyl-2,4-oxazolidinedione boiled at 122–127° at 0.5 mm. After three crystallizations from ether–petroleum ether it formed thin blades which melted at 53–55°. *Anal.* Calcd. for $C_6H_9NO_3$: N, 9.80. Found: N, 9.63.

5-Isopropyl-2,4-oxazolidinedione boiled at 118–119° at 1.5 mm., $n^{25}D$ 1.4671. *Anal.* Calcd. for $C_6H_9NO_3$: N, 9.80. Found: N, 9.87.

5-Methyl-5-*n*-propyl-2,4-oxazolidinedione is a thick, colorless oil; b. p. 115–118° at 1 mm., $n^{25}D$ 1.4583. *Anal.* Calcd. for $C_7H_{11}NO_3$: N, 8.91. Found: N, 8.75.

N-Methylation was carried out with dimethyl sulfate as described before[1] except that the use of methanol as solvent gave more consistent yields.

Introduction of higher alkyls by reaction of silver salts with alkyl iodides[1] gave poor yields and the method was soon abandoned.[6] Better results were obtained by preparing the potassium salt of the 2,4-oxazolidinedione in Cellosolve (glycol monoethyl ether) and heating with the appropriate halide. Chlorides, bromides and iodides were substantially equivalent, although with iodides it was found best to add the halide slowly to the cooled solution or suspension of the salt.

(1) Spielman, THIS JOURNAL, **66**, 1244 (1944).

(2) Everett and Richards, *J. Pharmacol.*, **81**, 402 (1944).

(3) Lennox, *J. Am. Med. Assoc.*, **129**, 1069 (1945); Perlstein and Andelman, *J. Pediatrics*, **29**, 20 (1946).

(4) Microanalyses by E. F. Shelberg. Compound 7 was made by W. B. Brownell. We are indebted to A. H. Smith, Jr., for technical assistance.

(5) Stoughton, THIS JOURNAL, **63**, 2376 (1941).

(6) Hook, *Nature*, **160**, 610 (1947), in a note which has just come to our attention has shown that the silver salt method may lead to O-alkylation.