ACTA CHEMICA SCANDINAVICA 5 (1951) 630–637

Determination of 4-Chloro-2-methylphenoxyacetic Acid in a Multicomponent System by Isotope Dilution Analysis

POUL SØRENSEN

Central Laboratory, Sadolin & Holmblad A/S, Copenhagen, Denmark

In this journal Sjøberg [1] described a method for determination of 4-chloro-2-methylphenoxyacetic acid (4K-2M) using infrared spectrometry. Grabe [2] has elaborated a similar method based upon ultraviolet spectrometry.

The advantages of isotope dilution are that no impurities will disturb determination and the procedure is quite simple. The accuracy is about $\pm$ 1.5 %, *i. e.* about the same as that attained by the infrared spectrophotometric method.

Isotope dilution technique is generally known [3]. Consider a crude sample which is to be analyzed for one of its constituents X. To the sample is added a known quantity of radioactive X, with a known specific activity (= activity per unit weight). From the mixture some pure X (or X of a known purity) should in one way or the other be isolated and the specific activity of this is determined. The ratio of the final and initial specific activities depends on how much the radioactive X has been diluted by the amount of inactive X present in the original sample, and from the measured quantities this amount can be calculated.

Using this method for this investigation the principle becomes:

Radioactive 4K-2M is synthesized from radioactive chlorine (Cl^{36}). A solution of this active 4K-2M is prepared with a content of y mg of 4K-2M per mg solution. The specific activity of the solution we call x (counts per minute per mg solution). Suppose a mg of active solution is mixed with a sample containing b mg of (inactive) 4K-2M. Pure 4K-2M isolated from the mixture will give the specific activity:

$$c = \frac{a \cdot x}{b + a \cdot y} \text{ counts per minute per mg}$$

Carrying out the same experiment with corresponding A and B mg and calling the final specific activity C, we have:

$$C = \frac{A \cdot x}{B + A \cdot y}, \text{ and by division}$$

$$r = \frac{C}{c} = \frac{A}{a} \cdot \frac{b + ay}{B + Ay} \quad \text{or}$$

$$B = \frac{1}{r}\frac{A}{a} \cdot b + A \cdot y \cdot \frac{1 - r}{r}$$

A standard sample of pure 4K-2M is made from a known amount b mg of 4K-2M to which is added a mg of active solution.

In the above expression B is the only unknown quantity. Starting with a sample containing an unknown amount B mg of 4K-2M, adding A mg of active solution, isolating a final sample of pure 4K-2M and measuring $r =$ the ratio of specific activity of the final sample and of the standard sample, the formula gives the value of B.

Instead of isolating 4K-2M a derivative of the compound may be isolated. If the standard sample is the same derivative the formula is still valid.

Is it only possible to isolate 4K-2M (eventually a derivative) with a known purity P %, the expression which determines B will be (with an approximation giving no practical difference in B):

$$B = \left(\frac{1}{r}\frac{A}{a} \cdot b + A \cdot y \cdot \frac{1 - r}{r}\right) \frac{P}{100}$$

Here it should be emphasized that a high purity of the radioactive 4K-2M is not necessary. Contamination of the radioactive 4K-2M with other radioactive substances may give rise to errors, but this source of error is eliminated by preparing the active solution with an acceptably high content of these components in inactive form. When isolating pure 4K-2M derivative by the procedure of the analysis, the radioactive contaminants are removed together with inactive species of the same compounds.

EXPERIMENTAL

The investigation was carried out with mixtures containing the same constituents as in Sjøberg's nvestigation, that is in addition to 4K-2M: 6-chloro-2-methylphenoxyacetic acid (6K-2M), 4,6-dichloro-2-methylphenoxyacetic acid (4,6K-2M) and 2-methylphenoxyacetic acid (2M).

Preliminary experiments showed that isolation of 4K-2M as an acid of known purity from a mixture of the mentioned constituents could not be done simply.

Experimenting with different derivatives it was found that the anilide of 4K-2M had practically ideal properties for the purpose. The anilide is easy to prepare, easy to gain in a nearly pure state by recrystallization and the purity can be determined with good accuracy by the melting point.

Melting points are determined by a capillary method in a Hershberg apparatus [4]. The tube is made from conventional glass capillary with a diameter about 1—1.5 mm. The middle of this capillary is drawn into an even narrower capillary about 20—30 mm long and about 0.1—0.2 mm diameter. The middle of the narrowed portion of the capillary is then divided in a flame so that two tubes are produced from one capillary. The sample is placed in the narrow end of the tube. The temperature at which a meniscus forms in the narrow end is taken as the melting point. Additional temperature values may be observed where „sintering" occurs and where *all* the substance melts. The bath is heated at a rate of about 0.1° C per minute in the vicinity of the expected temperatures. The difference in melting points of two samples is determined by placing the two tubes in the Hershberg-apparatus at the same time; the two tubes being made from the same capillary. The difference in melting points (meniscus points) is determined with an accuracy of ± 0.1° C.

Limits given for melting points refer to the sintering and meniscus point.

Radioactivity was measured with a Madsen-tube (bellshaped end window-counter, window thickness = 3 mm/cm^2, back ground = about 20 counts per minute [5]) and a Brüel and Kjær electronic counter 6501.

About 50 mg of the fine crystalline sample was placed in an aluminium dish and weighed. The measured number of counts per minute is corrected for background and self-absorption. Application of the sample in a uniform layer in the aluminium dish is done by suspending the powder in methanol on the dish and smoothing the sample with a nickel-spatula during the evaporation of the methanol. Finally the sample is dried under an infra-red lamp and reweighed.

The accuracy of determining activity is limited by the counting error. The error of the total count depends on the counting time. When counting samples of the activity as used in these experiments (500—1 000 counts per minute) for about ½—1 hour the standard deviation of the number of counts will be about 0.7 %, *i. e.* the ratio r (of the two samples specific activities) is determined with a standard deviation of about 1 %. In comparison with this error, the errors arising from corrections for background and selfabsorption, weighing of the sample and application on the aluminium dish are small and may be ignored.

MATERIALS

The phenoxyacetic acids were prepared by methods similar to Sjøberg's. Another way used to get pure products was purifiing the anilide followed by saponification.

Table 1 gives the melting points and solubilities of the acids used.

The anilides were prepared by refluxing the acid in question with 2—3 times as much aniline (b. p. 184—185) one hour, precipitating with an excess of 4 N HCl, filtering and

Table 1. Properties of phenoxyacetic acids.

Acid	m. p. C°	Solubilities at 25° C mg per 100 ml of solution		
		water	carbon tetrachloride	benzene
4K—2M	119.6—119.8	75	600	3 100
2M	155.1—155.3	60	50 [1]	
6K—2M	108.9—109.3	220		
4,6K—2M	187.6—188.0	8	50	200

[1] From Sjøberg's investigation.

Table 2. Melting points and analyses of anilides of phenoxyacetic acids.

Anilide of	m. p. C°	Microanalyses (by F. Limborg)							
		C %		H %		Cl %		N %	
		calc.	found	calc.	found	calc.	found	calc.	found
4K—2M	129.8—130.0 [1] 129.5—129.7 [2]	65.4	65.7	5.12	4.9	12.9	12.9	5.08	5.3
2M	108.2—108.4	74.7	74.3	6.27	5.8	0		5.81	5.7
6K—2M	78.0— 78.3	65.4	65.5	5.12	5.0	12.9	12.9	5.08	5.0
4,6K—2M	100.8—101.1	58.1	58.1	4.23	4.2	22.9	22.9	4.52	4.4

[1] Recrystallized from methanol.
[2] Recrystallized from 61 % ethyl alcohol.

Table 3. Solubilities of anilides of phenoxyacetic acids.

Anilide of	Solubility at 25° C mg per 100 ml of solution	
	abs. ethyl alcohol	61 % ethyl alcohol
4K—2M	1 100	150
2M	2 600	700
6K—2M	10 000	1 500
4,6K—2M	3 300	300

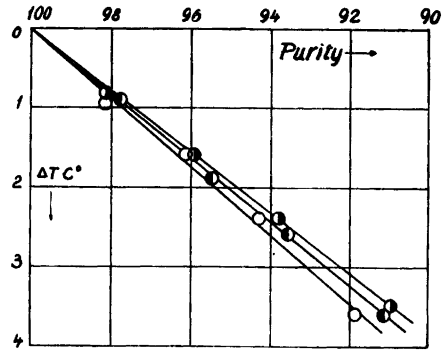

Fig. 1. Melting point depression of 4K-2M-anilide.

◑ *4,6K-2M-anilide*
◐ *6K-2M-anilide*
○ *2M-anilide*

Table 4. Purity determination from melting points.

Deviation of m. p. ΔT C°	0.1	0.2	0.3	0.4	0.5	0.6
Purity	99.8	99.5	99.3	99.0	98.8	98.5

washing with water and recrystallization from abs. ethyl alcohol or from 61 % alcohol (= 2 vol. alc. + 1 vol. water).

Tables 2 and 3 gives analytic data, melting points and solubilities of the anilides.

Radioactive 4K-2M was prepared by chlorination of 2-methylphenoxyacetic acid with radioactive chlorine *. (Cl^{36}, half life = $2 \cdot 10^6$ years, β-emitter, 0.66 Mev.) The starting material was about 10μ-Curie NaCl as a dilute aqueous solution. The chloride was precipitated as AgCl and dried. From AgCl the chloride was transformed to elementary chlorine by oxidation with potassium dichromate in sulfuric acid after Baubigny and Chavanne [6], with a yield of about 95 %. 1.03 g of methylphenoxyacetic acid dissolved in 10 g of acetic acid at 100° C was chlorinated by the chlorine produced from 1.947 g of active AgCl. The chlorinated solution was diluted 15 times with water and after heating to clear solution, crystallized when cool. Crystals of active 4K-2M were separated from the mother liquor and dried. Yield: 0.964 g, m. p. 118.5—119.0, estimated purity: 95 %.

The HCl evolved by the process together with other chlorine-containing fractions were transformed back to AgCl. Yield for consumed chlorine was about 80 %.

Preparation of radioactive solution: 420 mg of active 4K-2M, 198 mg of 4,6K-2M and 221 mg of 6K-2M were dissolved in 20 ml of ethyl alcohol and 6 ml of 1 *N* NaOH were added. Water was added to total weight 40.0 g. The content of 4K-2M is about 1 % that is $y \cong 0.010$ mg of 4K-2M per mg solution. Specific activity (x) about 4 counts per minute per mg solution when counting with the afore-mentioned apparatus.

---

* The Cl^{36} samples used were from the U. S. Atomic Energy Commission, Oak Ridge.

Purity determination of 4K-2M-anilide

Mixtures of the anilide of 4K-2M and an anilide of other phenoxyacetic acids were prepared by dissolving these in acetone and evaporating to dryness. Fig. 1 shows the depression of the melting point (meniscus point) of 4K-2M-anilide by different contents of anilides of the other acids. By using the curves for purities above 99 % the different curves give practically the same corrections. Table 4 gives the purity at different melting point-differences (by extrapolation).

ANALYTICAL PROCEDURE

A quantity of the product to be analyzed, which is estimated to contain about 200 mg of 4K-2M is weighed. 1 ml of active solution is added and accuratly weighed (A mg). After eventually dissolving in NaOH the phenoxyacetic acids are precipitated by 4 N HCl and isolated by filtering or decanting.

1.5 ml of aniline is added and in a test-tube provided with a "cold finger" is refluxed for an hour. Some waterdrops from the wet acids will condense on the "cold finger" and may be removed with a piece of filterpaper. After cooling add 10 ml of 4 N HCl, boil again a moment and cool off. The anilides are washed with water, the supernatant being filtered to reduce loss. The wet crude anilides are recrystallized first from 5 ml of abs. ethyl alcohol and then two or more times from 61 % alcohol until the difference in melting point from that of the standard sample is less than 0.5° C that is to say a purity above 99 %. For technical products usually two or three recrystallizations from 61 % alc. are enough. After the last recrystallization the product is washed with about 1 ml of methanol and dried under an infra-red lamp. The yield of 4K-2M-anilide is about 100—150 mg. The specific activity as a ratio of the standard sample (prepared in the same way, but from a known amount (b mg) of pure 4K-2M) is measured ($= r$). From the melting point the purity (R %) is determined and the amount (B mg) of 4K-2M in the original sample is calculated.

RESULTS

Following samples were analyzed:

Table 5.

Sample	Composition							
	4K-2M		2M		6K-2M		2M-4, 6K	
	mg	%	mg	%	mg	%	mg	%
A	400.7	66.7	40.2	6.7	119.5	19.9	39.8	6.7
B	292.4	59.5	40.2	8.2	119.5	24.3	39.8	8.1
C	256.2	72.0	20.1	5.6	59.8	16.8	19.9	5.6

In the case of sample A, several recrystallizations were performed and between each recrystallization from 61 % alcohol, the melting point and activity were determined.

Table 6. Analyses of known mixtures (see Table 5).

Sample	Deviation of m. p. from standard ΔT C°	Purity R %	Active solution added A (a) mg	Specific activity in relation to standard r	Amount of 4K − 2M		Deviation %
					found B mg	calc. mg	
Standard	0	100.0	1855.8	1.000		395.0	
A	1.1	97.5	1876.3	0.973	401	400.7	+ 0.1
A	0.5	98.8	1876.3	0.991	398	400.7	− 0.7
A	0.3	99.3	1876.3	0.997	399	400.7	− 0.4
A	0.2	99.5	1876.3	0.999	398	400.7	− 0.7
B	0.5	98.8	1838.0	1.296	294	292.4	+ 0.6
C	0.3	99.3	924.4	0.771	256	256.2	− 0.1

DISCUSSION

By estimation of the accuracy of the method it is obvious that all other uncertainties may be ignored in comparison with the uncertainty of r. The quantity y is rather inaccurratly determined but the term with y only represents about a per cent or less of the total value.

The purity R may give rise to errors but if the purity determined by melting point is above 99 % it is not probable that impurities even though unknown will lead to inaccurate results.

SUMMARY

4-Chloro-2-methylphenoxyacetic acid has been determined in mixtures with other phenoxyacetic acids by an isotope dilution method.

The standard deviation of the result is calculated to be about 1 %, *i. e.* the real value is with a 90 % probability between the determined value $\pm$ 1.64 %.

The author gratefully acknowledges the opportunity of carrying out the radioactivity measurements at the Zoophysiological Laboratory of the University of Copenhagen.

Dr. A. Hobgen kindly revised the English text.

REFERENCES

1. Sjøberg, B. *Acta Chem. Scand.* **4** (1950) 798.
2. Grabe, E. *Acta Chem. Scand.* **4** (1950) 806.
3. Yankwich, P. E. *Anal. Chem.* **21** (1949) 318.
4. Hershberg, E. B. *Ind. Eng. Chem., Anal. Ed.* **8** (1936) 312.
5. J. Ambrosen, B. Madsen, J. Ottesen, and K. Zerahn *Acta Physiol. Scand.* **10** (1945) 195.
6. Baubigny, H., and Chavanne, G. *Compt. rend.* **139** (1903) 1198, **138** (1904) 85.

Received March 1, 1951.