phenyl ring substitutions in the reference compound, α-methoxyphenylacetic acid, on the other hand, exhibited moderate or marked plant-regulating activity as did *m*-nitro-, O, substitution. These compounds were not exuded by the roots of treated plants in biologically detectable amounts. Ring positions 3 and 4, therefore, show limited flexibility in the type of substituent that can be used to replace the hydrogen without interfering with the ability of the roots to exude the compound. α-Aminophenylacetic acid, the ethyl ester of α-cyanophenylacetic acid, α-methoxydiphenylacetic acid, α-phenylbutyric acid, *p*-isopropyl-α-methoxyphenylacetic acid (ammonium salt), and 4-methyl-α-methoxyphenylacetic acid (ammonium salt) were only slightly active or were inactive and these compounds could not, therefore, be critically evaluated for root exudation.

α - 2,3 - Trimethoxyphenylacetic acid (ammonium salt), Q, possessed regulating activity equal to or greater than that of α-methoxyphenylacetic acid, but lacked the root-exudation characteristic. In contrast, α-3,4-trimethoxyphenylacetic acid, R, possessed no detectable regulating activity and the α-*o*-dimethoxy-substituted compound, P, tested possessed only slight activity.

With respect to α-methoxyphenylacetic acid, loss of activity also resulted when a methanethiol group or a chlorine atom was substituted for the methoxy group in the side chain. Reduced activity likewise occurred when a methyl group was substituted in the *o*-, *m*-, and the 2,3,6-positions on the ring. Alkoxy groups, except 2,3-dimethoxy-, reduced the regulating activity when used as the following ring substituents: *o*-ethoxy, *o*-methoxy, 3,4-diethoxy, and 3,4-dimethoxy. The 3,4-methylenedioxy substitution reduced the activity slightly. Complete loss of or a decrease in regulating activity resulted when the phenyl ring was replaced with the following structures: a methyl group, the 2-furyl group, 2-thienyl group, or the α-naphthyl group.

The data presented in this paper show that of those compounds tested, relatively few changes in either the aromatic or the aliphatic portion of α-methoxyphenylacetic acid resulted in compounds that retained both the marked plant-regulation and root-exudation characteristics of the acid.

Acknowledgment

Wilkinson Reeve, Chemistry Department, University of Maryland, synthesized all compounds reported, except the ethyl ester of α-cyanophenylacetic acid which was obtained from the Chemical-Biological Coordination Center of the National Research Council and the optical isomers of methoxyphenylacetic acid which were obtained from W. A. Bonner.

Literature Cited

(1) Linder, P. J., Craig, J. C., Jr., Cooper, F. E., Mitchell, J. W., J. AGR. FOOD CHEM. **6,** 356 (1958).
(2) Linder, P. J., Craig, J. C., Jr., Walton, T. R., *Plant Physiol.* **32,** 572 (1957).
(3) Mitchell, J. W., Preston, W. H., Jr., *Science* **118,** 518 (1953).
(4) Preston, W. H., Jr., Mitchell, J. W., Reeve, W., *Ibid.*, **119,** 437 (1954).
(5) Reeve, W., Christoffel, I., *J. Am. Chem. Soc.* **72,** 1480 (1950).
(6) Reeve, W., Pickert, P. E., *Ibid.*, **79,** 1932 (1957).

Received for review July 9, 1959. Accepted September 2, 1959.

SULFUR DETERMINATION

Nitric-Perchloric Acid Oxidation for Sulfur in Plant and Animal Tissues

W. M. SHAW

University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.

The available methods for the determination of total sulfur in plant and animal tissue were found to be either cumbersome or of low degree of precision. A simple procedure utilizing nitric and perchloric acid oxidation was developed, which gives precise and accurate results for sulfur comparable with those obtained by the AOAC magnesium nitrate method on a great variety of plant and animal products, and the amino acids, cystine, cysteine, and methionine. Sulfur recoveries in standard materials were within 2% of theory. The same sample preparation can serve for the determination of calcium, magnesium, potassium, sodium, and phosphorus, as well as sulfur. The sulfate was determined gravimetrically as barium sulfate, but the prepared solution can be adapted for the indirect flame spectrophotometric or any other desired method of sulfur determination.

A SURVEY OF RECENT LITERATURE in the agronomic and botanical fields showed that for total sulfur in plants, the AOAC magnesium nitrate method (*1*) was in common use. This presents a rather anomalous situation in view of the fact that the wet-ashing of plant materials by nitric-perchloric acids has found wide acceptance in the mineral analysis of plants, which in some instances has included determinations of sulfur. The long-drawn-out digestion technique frequently used in studies on sulfur undoubtedly has been the greatest deterrent to the use of the nitric-perchloric acid procedure for sulfur. On the other hand, some workers reported satisfactory sulfur results on plant materials using short-time digestion periods.

The objective of the present study was to re-examine critically the factors of time and temperature in the nitric-perchloric acid oxidation procedure with a view to redefining the conditions of this procedure, so that total sulfur could be determined in the same sample preparation that would be used for the mineral analysis of organic materials. Any special sulfur procedures, and particularly those that introduce high salt concentrations or analytical reagents that would interfere with the determinations of the mineral elements in the sample, would be outside the present objectives.

Experimental

The experimental work was designed primarily to define adequately the time and temperature factors in the final stage of perchloric acid digestion for routine oxidation of plant and biological materials in 1-gram quantities. Flexibility in operating conditions, such as size of sample, reaction container, and heating devices, greatly influences the accuracy as well as the acceptability of a

procedure. Because of its greatest resistance to oxidation, methionine was chosen as the test material in all the preliminary investigations.

Procedure

Weigh a 1-gram sample of plant or animal tissue or 0.1 gram of sulfur compound. Transfer the sample to a 100-ml. Kohlrausch borosilicate glass flask, and wash down dust particles with a little water. Introduce 10 ml. of equal parts of nitric and 70% perchloric acids and swirl the contents until the material is completely wetted and in suspension. Light the two burners on one end of the gas hot plate. Place the flasks toward the warm end of the plate, apply heat cautiously, and guard against excessive foaming. (An electric plate may be used, but the gas hot plate was found more convenient.) Increase the heat, and maintain the digestions at active, but not excessive boiling; continue until white fumes appear. Maintain the boiling for 1 hour after the digested solution has assumed a pale greenish yellow color. Swirl the contents twice at 20-minute intervals. Remove the flasks from the hot plate, introduce about 40 ml. of distilled water, and heat at boiling temperature for 15 minutes. Filter off insoluble matter and wash with hot water. (The filtrate may be used for either gravimetric or other methods (*7*) of sulfate determination.)

Gravimetric Sulfur Determination. Neutralize the filtrate with (1 + 1) ammonia (about 7 ml.) and acidify with 2 ml. of dilute (1 + 1) hydrochloric acid. Dilute to about 100 ml., heat to boiling, and precipitate the sulfate with 10 ml. of a 5% barium chloride solution, in the usual manner. Digest the precipitate 1 to 2 hours on the hot plate and allow to stand overnight, at room temperature. Filter the barium sulfate on a porous porcelain crucible of medium fineness, using slight suction (5 to 10 cm. of mercury). Quantitatively transfer the precipitate to the crucible and wash six times with hot water. Ignite in an electric furnace at 650° C., or just at red heat. Cool in the desiccator and weigh as barium sulfate. Use the factor 0.1374 to convert the barium sulfate weight to that of sulfur.

The gravimetric determination was used only as the best way of establishing the reliability of the oxidation procedure, but without the intention of making it a necessary adjunct of the oxidation procedure.

Preliminary Studies

The preliminary studies, dealing with time, temperature, digestion, size, and shape of container were based on sulfur recovery from methionine and are embodied in Tables I and II. The 2-hour sulfur recovery (Table I) amounted to 95.4% of theory and 0.9% short of the recovery reported by Evans and St. John (*2*). Digestion at periods longer than 2 hours failed to give a more complete sulfur recovery from the methionine sample.

Beakers *vs.* Flasks as Reaction Containers. The results in groups A and B of Table II show the comparative recoveries of methionine sulfur from 12 digestions each in 150-ml. beakers and in 100-ml. Kohlrausch flasks. The digestions in flasks B showed an increase of 1.6% in sulfur recovery and one fourth the standard deviation as compared with those obtained with A. The greater deviations among beaker results were due to greater variation in the glass thickness, which caused some to boil briskly, while others were just at a simmer.

Table I. Recovery of Sulfur from Methionine Using Different Periods of Digestion in Perchloric Acid

Digestion in $HClO_4$, Hours							
2	4	6	8	12	16	20	24
Sulfur Recovered, %[a]							
20.5	20.5	20.4	20.3	20.4	20.4	20.6	20.6
20.4	20.3	20.6	20.1	20.1	20.3	20.5	20.8
20.6	20.3		20.6	20.8	20.9	20.4	
	20.3		20.9	20.6	21.2	20.0	
			21.0				
20.5	20.40	20.50	20.58	20.38	20.56	20.30	20.70
Std. dev.	±0.10	±0.10	±0.30	±0.23	±0.28	±0.22	±0.10

[a] Theoretical value 21.49%; 100-mg. sample used.

Table II. Total Sulfur in Methionine Using Different Types of Reaction Container, Times, and Temperature of Digestion[a]

Group	Reaction Container and Digestion Temp.		Duration of Digestion, Hours: In HNO_3	In $HClO_4$	Sulfur, % 1	2	3	4	5	6	Av. ± Std. Dev.	Recovery, % of Theory ± Std. Dev.
A	150-ml. Griffin beaker, 198–203° C.	(a)[b]	2	2	21.19	21.24	20.77	21.20	20.68	20.95	21.00 ± 0.24	
		(b)	2	2	20.47	20.57	19.98	19.87	20.72	19.72	20.22 ± 0.42	
										Group av.	20.51	95.4 ± 1.5
B	100-ml. Kohlrausch flask, 198–203° C.	(a)	2	2	20.75	20.75	20.80	20.82	20.65	20.95	20.79 ± 0.09	
		(b)	2	2	20.88	20.88	20.83	20.71	20.72	20.77	20.80 ± 0.07	
										Group av.	20.80	97.0 ± 0.
C	100-ml. Kohlrausch flask, 198–203° C.	(a)	3	2	21.10	21.12	21.19	21.19	20.98	21.27	21.14 ± 0.09	
		(b)	3	2	20.68	20.91	21.10	21.17	21.09	21.02	21.00 ± 0.18	
										Group av.	21.07	98.0 ± 0.6
D	100-ml. Kohlrausch flask, 198–203° C.	(a)	1	2	21.02	20.91	21.08	20.99	20.88	21.12	21.00 ± 0.09	
		(b)	1	2	21.06	21.02	20.50	20.95	20.79	20.69	20.84 ± 0.21	
										Group av.	20.92	97.3 ± 0.7
E	100-ml. Kohlrausch flask, 198–203° C.	(a)	1	1	21.16	21.19	20.75	20.93	21.15	18.78	20.66 ± 0.93	
		(b)	1	1	20.99	20.98	21.13	21.02	21.02	18.96	20.68 ± 0.84	
										Group av.	20.67	96.1 ± 4.1
F	100-ml. Kohlrausch flask, 185–188° C.		1	2	18.00	20.47	7.17	20.82	20.86	11.29	16.44 ± 3.7	
G	100-ml. Kohlrausch flask, 150–160° C.		2	5	0.55	2.61					1.58 ± 1.45	

[a] A 100-mg. sample was used.
[b] (a) and (b) denote sets of determinations made on different days.

Table III. Comparison of Magnesium Nitrate and Nitric-Perchloric Acid Oxidation Procedures for Determination of Total Sulfur in Plant and Animal Materials

No.	Material	A, AOAC Method N	A, AOAC Method S, % ± std. dev.	B, Nitric-Perchloric Acid Digestion N	B, Nitric-Perchloric Acid Digestion S, % ± std. dev.	Diff. B − A	t at 5% Probability Level: Calculated value	t at 5% Probability Level: Critical value
			Forages and Others					
1	Soybean hay	3	0.206 ± 0.000	9	0.213 ± 0.010	0.007	1.105	2.228
2	Wheat straw	5	0.074 ± 0.008	9	0.088 ± 0.005	0.014[a]	2.715	2.179
3	Sudangrass 30	4	0.155 ± 0.009	4	0.155 ± 0.011	0.000	1.414	2.447
4	Ryegrass 93-1	3	0.341 ± 0.012	7	0.331 ± 0.003	−0.010	2.195	2.306
5	Crimson clover	4	0.207 ± 0.015	6	0.231 ± 0.007	0.024[a]	2.760	2.306
6	Red clover	3	0.148 ± 0.000	12	0.147 ± 0.004	−0.001	1.444	2.131
7	Corn plant	3	0.151 ± 0.006	4	0.172 ± 0.005	0.021[a]	3.700	2.571
8	Corn leaves	3	0.226 ± 0.003	3	0.222 ± 0.005	−0.004	0.999	2.776
9	Cotton leaves and petioles	3	0.842 ± 0.004	3	0.827 ± 0.015	−0.015	1.611	2.776
10	Tobacco	6	0.631 ± 0.006	7	0.628 ± 0.007	−0.003	0.438	2.179
			Vegetables					
11	Tomato plant	2	0.653 ± 0.003	3	0.676 ± 0.005	0.023[a]	3.830	3.182
12	Broccoli	5	0.963 ± 0.016	5	0.938 ± 0.013	−0.025[a]	2.725	2.306
13	Cabbage	3	1.749 ± 0.005	5	1.715 ± 0.020	−0.034[a]	2.855	2.447
14	Turnip greens	6	1.143 ± 0.028	5	1.150 ± 0.013	0.007	0.369	2.365
			Seeds					
15	Soybeans	8	0.370 ± 0.010	3	0.380 ± 0.018	0.010	1.180	2.262
16	Corn	6	0.132 ± 0.008	3	0.144 ± 0.007	0.012	2.232	2.365
17	Wheat	6	0.151 ± 0.011	3	0.160 ± 0.006	0.009	1.087	2.365
18	Oats	6	0.186 ± 0.008	3	0.197 ± 0.015	0.011	1.728	2.365
19	Barley	6	0.121 ± 0.008	3	0.135 ± 0.021	0.014	1.488	2.365
20	Cottonseed meal A	3	0.462 ± 0.003	6	0.470 ± 0.017	0.008	0.870	2.365
21	Cottonseed meal B	5	0.541 ± 0.007	3	0.532 ± 0.006	−0.009	1.836	2.447
			Animal Products					
22	Blood meal	5	0.563 ± 0.018	5	0.554 ± 0.020	−0.009	0.710	2.306
23	Feather meal	5	1.766 ± 0.063	5	1.780 ± 0.012	0.014	0.480	2.306
24	Casein	5	0.698 ± 0.009	5	0.685 ± 0.007	−0.013[a]	2.430	2.306
25	Methionine	5	19.64 ± 0.230	5	21.00 ± 0.094	1.360[a]	11.680	2.306
26	Cystine	5	26.50 ± 0.219	5	26.12 ± 0.130	−0.380[a]	3.336	2.306
27	Cysteine hydrochloride, hydrate	3	18.34 ± 0.173	3	18.16 ± 0.136	−0.180	1.161	2.780

[a] Statistically significant difference at 5% probability level.

Furthermore, active boiling in some of the beakers caused considerable splashing, which sometimes resulted in mechanical loss. The borosilicate glass Kohlrausch flasks are of more uniform thickness, the boiling is more regular, and the condensate flows down gently on the sides of the flask, whereas in beakers the condensate drops suddenly into the midst of the hot digestate.

Time by Digestion Stages. Previous studies placed great emphasis on the slow rate for the entire digestion process, which includes the distinctly nitric acid oxidation, the intermediate stage, and the perchloric acid reaction, boiling at 160° to 203° C.

The digestions of groups B, C, and D of Table II were all conducted at the final temperature of 198° to 203° C., but differed as to the time alloted for the perchloric acid step and the preceding steps denoted as nitric acid reaction. The sulfur recoveries ranged from 97 to 98% of theory, with standard deviations of 0.4 to 0.7%. The 1-hour perchloric acid digestion (group E) gave a sulfur recovery of 96.1% and a standard deviation of 4.1%. The deviations are due to a single low value in each set of six individual values. The two low values might be considered as accidental, but under the circumstances, the 2-hour digestion in perchloric acid must be accepted as giving the highest recovery and greatest precision for methionine.

Effect of Temperature of Perchloric Acid Digestion. The importance of proper digestion temperature has been indicated earlier. The boiling temperature of perchloric acid ranging from 198° to 205° C. was generally used for the oxidation of methionine. The data groups F and G (Table II) are given to show how the results might be affected by digestions in perchloric acid at other than at the boiling temperature. The digestions in group F were maintained at incipient boiling (190° C.), whereas those in group G were carried to copious fumes and colorless solution at 150° to 160° C.

The temperatures of F and G (Table II) satisfied the common requirements of digestion to the point of "fuming and a colorless solution," but gave results that were erratic and very low.

Analyses of Plant and Animal Tissues

The data of Table III represent a test of accuracy on 27 materials for total sulfur by the nitric-perchloric acid oxidation—2 hours in perchloric acid—as compared with the AOAC magnesium nitrate method. The differences between the means of the two methods are given in column $B - A$ in which the negative sign indicates a lower value by the nitric-perchloric acid method. In a total of 27, nine comparisons show significant mean differences according to t test at 95% probability level. However, five out of the number deviate positively, while the remaining four deviate negatively.

On the basis of 21.5% theoretical sulfur content in methionine, the recovery by the magnesium nitrate method was 91.4 ± 1.2% standard deviation, whereas that by the nitric-perchloric acid method was 97.7 ± 0.5% standard deviation. No such discrepancy, however, was found in the results for casein by the two methods, although the sulfur of this material is about 80% methionine (*4*). McChesney and Banks (*5*) obtained for methionine after 1-hour digestion in the perchloric acid phase, 99.2% recovery ± 3.9% standard deviation. Evans and St. John (*2*) after a 16-hour digestion in perchloric acid obtained a recovery of methionine sulfur of 96.3%.

On the basis of 26.7% theoretical sulfur content, the recoveries of cystine sulfur by the magnesium nitrate and nitric-perchloric acid methods were 99.2 and 98.0%, respectively. Evans and St. John (*2*), using the prolonged procedure, obtained a cystine sulfur recovery of 95.5%. McChesney and Banks (*5*) obtained a cystine sulfur recovery of 98.6 ± 1.1%.

Table IV. Total Per Cent Sulfur in Plant Materials Using 1- and 2-Hour Digestions in Perchloric Acid

Material	1 Hour					2 Hours					1–2-Hour Mean Diff.	Calcd. t Value[a]
	Determinations			Mean	Std. dev.	Determinations			Mean	Std. dev.		
	1	2	3			1	2	3				
Broccoli	0.967	0.956	0.973	0.965	0.005	0.944	0.958	0.932	0.945	0.013	0.020	2.50
Turnip greens	1.173	1.172	1.179	1.175	0.004	1.150	1.162	1.164	1.159	0.008	0.016	3.16
Cotton leaves	0.834	0.860	0.852	0.849	0.013	0.818	0.818	0.845	0.827	0.016	0.022	1.86
Corn leaves	0.228	0.228	0.224	0.227	0.002	0.225	0.217	0.225	0.222	0.005	0.005	1.80
Tobacco	0.635	0.632	0.629	0.632	0.003	0.640	0.629	0.628	0.632	0.007	0.000	0.00
Cottonseed meal, B	0.539	0.534	0.530	0.534	0.004	0.536	0.525	0.536	0.532	0.006	0.002	0.57
Blood meal	0.547	0.558	0.559	0.555	0.009	0.563	0.561	0.561	0.562	0.002	−0.007	2.14
Feather meal	1.772	1.770	1.800	1.781	0.023	1.722	1.782	1.775	1.780	0.046	0.001	0.05
Casein	0.692	0.680	0.692	0.688	0.008	0.684	0.687	0.680	0.684	0.005	0.004	0.98

[a] Calculated according to Youden (*8*). Critical *t* value for 4 degrees of freedom at 5% probability level = 2.78.

On the basis of 18.26% theoretical sulfur content, the recoveries of cysteine sulfur were 100.4 and 99.5% by the two methods, respectively.

The differences between methods for samples other than the high sulfur-containing amino acids range from 0.010 to 0.034% sulfur. The greatest percentage differences (10 to 12%) occur among the low sulfur-content forage samples. In the vegetable group, the differences were 2 to 3%; in the animal products, they were 1 to 2%; and in the seed group, the differences were negligible.

On the basis of the comparative sulfur determinations (Table III) on 24 vegetable and animal materials, and three sulfur-containing amino acids by the AOAC magnesium nitrate and the nitric-perchloric acid methods of oxidation, it is concluded that the nitric-perchloric acid method, using a 2-hour boiling digestion in perchloric acid, may be relied upon to yield total sulfur results within an accuracy of 1 to 2% of theoretical values. With low sulfur vegetation (<0.2% S), the precision may be improved by increasing the sample size to 2 grams.

One-Hour *vs.* Two-Hour Digestion of Plant and Animal Tissues. A 2-hour digestion in perchloric acid as used in the determinations of Table III was based on indications of higher precision in the 2-hour digestion of methionine (Table II). Because of the exceptional properties of methionine, additional tests with natural plant and animal products were conducted. The data of Table IV represent results of such tests on nine materials. There is nothing in the results with the nine materials to indicate lower yields or lower precision in the 1-hour digestion as compared to those from the 2-hour digestion. On the contrary, for the 1-hour digestion the average coefficient of variation is 0.55%, whereas that for the 2-hour digestion is 0.78%. The mean differences tested between the two periods of digestion of the different materials show no significance by the *t* test at the 5% probability level.

When preceded by digestion in nitric acid or in a mixture of the two acids, a 1-hour digestion in perchloric acid is ample for the complete sulfur oxidation of 1-gram samples of any vegetable or animal tissue. Possibly, even a shorter time in perchloric acid might prove sufficient, but in view of the over-all time required for the entire procedure, a further one-half hour gain is of doubtful benefit. The 1-hour counting time begins with the boiling in perchloric acid, when the solution has assumed a pale greenish yellow color.

Table V. Total Sulfur in Acid-Decomposable Inorganic Sulfur Compounds Using HNO_3–$HClO_4$ Oxidation

Detn.	Sodium Thiosulfate, $Na_2S_2O_3 5H_2O$, %	Sodium Metabisulfite, $Na_2S_2O_5$, %	Sodium Sulfite, Na_2SO_3, %	Precipitated Zinc Sulfide, ZnS, %	Mascot Zinc, Concentrate, %
1	26.53	32.80	25.42	30.70	30.19
2	26.43	32.78	25.56	30.40	30.76
3	25.76	32.88	25.64	30.60	30.31
4			25.47	31.26	30.85
5			25.64	31.66	30.64
Mean	26.24	32.82	25.55	30.82	30.58
% of theoretical	101.66	97.30	100.42	93.70	101.10[a]
Std. dev.	0.42	0.05	0.199	0.63	0.29
Std. error of mean	0.24	0.03	0.085	0.28	0.13

[a] Based on determined 30.24% S by bromine oxidation analysis (*3*).

Table VI. Effect of Perchloric Acid Evaporation on Sulfate Recovery from K_2SO_4 in Presence or Absence of Calcium Ions

SO_4-S Taken, Mg.	Ca, Mg.	SO_4-S Recovered, Mg.				Av. Recovery, %
		1	2	3	Av.	
5	0	2.88	2.61	2.34	2.61	52.2
10	0	7.09	7.56	7.05	7.23	72.3
20	0	16.30	16.27	15.87	16.15	80.8
5	80	4.88	4.86	5.00	4.91	98.2
10	80	9.85	9.80	9.96	9.87	98.7
20	80	19.40	19.69	19.70	19.60	98.0
5	5	4.18	4.19	4.18	4.18	83.6
5	6.25	4.81	4.81	4.81	4.81	96.2
5	7.50	4.95	4.90	4.85	4.90	98.0
5	8.75	4.88	4.81	4.81	4.83	96.6
5	10.00	4.88	4.88	4.88	4.88	97.6
	Na					
10	200	8.13	7.39	7.69	7.74	77.4

Sulfur Retention in Nitric-Perchloric Acid Oxidation. An authoritative handbook on methods of agronomic research has disqualified the nitric-perchloric acid oxidation for sulfur determination in plants, on the alleged grounds that the wet digestion procedure does not quantitatively retain sulfur (*6*). No supporting evidence was cited, and investigations that satisfactorily utilized the wet digestion procedure were apparently disregarded.

Tests of sulfur retention were conducted with 0.1 gram of the following sulfur compounds: sodium thiosulfate, sodium metabisulfite, sodium sulfite, precipitated zinc sulfide, and a zinc mineral concentrate. The first three of these materials are soluble in water, and upon contact with acids give off sulfur dioxide; the last two are soluble only in acids and give off hydrogen sulfide. The solution of the precipitated sulfide in acid is immediate, while the solution of the mineral sulfide is gradual. The determinations were carried out in the usual manner except that the digestion was started first with nitric acid at room temperature. The results of the

sulfate determinations are given in Table V.

Sulfur recoveries of 100% were obtained from sodium sulfite, sodium thiosulfate, and the mineral zinc sulfide concentrate should assure the complete sulfur retention in the nitric-perchloric acid method of oxidation of biological materials.

Active boiling in perchloric acid of standard quantities of potassium sulfate for 1, 2, and 4 hours showed identical sulfate recoveries.

Effect of Perchloric Acid Evaporation on Sulfate Recovery. It is often necessary or desirable to evaporate the residual perchloric acid before precipitation of sulfates as barium sulfate. In such instances sodium chloride has been used as the fixative for sulfate. Hillebrand *et al.* (*3*) however, maintain that evaporation of sulfates in perchloric acid solution can be carried to dryness without loss of sulfuric acid, provided the solution contains a relatively large excess of an element such as calcium. The calcium excess necessary to obtain full recovery of sulfate after evaporation to dryness in perchloric acid of such materials as the sulfur containing amino acids was determined (Table VI).

Evaporation of potassium sulfate to dryness from perchloric acid solution showed losses of 50 to 20% of sulfate content. With calcium additions in the calcium to sulfur weight ratios of 1 to 1 up to 2 to 1, the maximum sulfur recovery (98%) was obtained with the 1.5 to 1.0 ratios. On the other hand, an addition of sodium as the chloride in the weight ratio of 20 to 1 showed a loss of 22.6% of the 10 mg. sulfate sulfur.

To retain all the sulfate upon evaporation in perchloric acid it is only necessary to have a calcium content to give a calcium-sulfur weight ratio of 1.5 to 1.0.

Literature Cited

(1) Assoc. Offic. Agr. Chemists, "Official Methods of Analysis," 8th ed., 1955.
(2) Evans, R. J., St. John, J. L., *Ind. Eng. Chem., Anal. Ed.* **16,** 630 (1944).
(3) Hillebrand, W. F., Lundell, G. E. F., Bright, H. A., Hoffman, J. F., "Applied Inorganic Analysis," 2nd ed., Wiley, New York, 1953.
(4) Horn, M. J., Jones, D. B., Blum, A. E., *J. Biol. Chem.* **166,** 321 (1946).
(5) McChesney, E. W., Banks, W. F., *Anal. Chem.* **27,** 987 (1955).
(6) Richards, L. A., U. S. Dept. Agr, Agricultural Handbook 60, 1954.
(7) Shaw, W. M., *Anal. Chem.* **30,** 1682 (1958).
(8) Youden, W. J., "Statistical Methods for Chemists," Wiley, New York, 1951.

Received for review January 29, 1959. Accepted August 24, 1959.

CESIUM-137 UPTAKE BY PLANTS

Factors Affecting Uptake of Radioactive Cesium by Lettuce, Grass, and Alfalfa

ERIC B. FOWLER and C. W. CHRISTENSON

Health Division, Los Alamos Scientific Laboratory, University of California, Los Alamos, N. M.

Cesium-137 forms about 6% of the products released on fission. It enters the biosphere through fallout and, hence, may find its way into plants and eventually into man. A study of soil factors which affect the uptake of cesium and its possible control are reported. An increase in the available potassium in the soil is reflected by a decrease in the cesium-137–potassium ratio in the plant. Discriminations by plants against cesium-137 were in the range of 300 to 1400, dependent upon the soil and the plant. Discrimination may, in part, be related to the ion exchange capacity of the soil and in part to the exchangeable potassium content of the soil. Potassium fertilizers may decrease the cesium-137–potassium ratio in the plant, but may mobilize cesium in some soils and thus render it more available to the plant.

An observed effect of potassium on the uptake of cesium by corn and millet has been reported by Menzel (*7*). A few widely varied discrimination factors against cesium by plants have been reported and reviewed by Langham and Anderson (*6*).

Cesium-137 forms about 6% of the products released on fission. It enters the biosphere through fallout and, hence, may find its way into plants and eventually into man. Unlike strontium, the biological half life of cesium is relatively short; however, a study of factors affecting the uptake of cesium-137 by plants is important because the widespread prevalence of this isotope in the biosphere effects its continued intake by man.

This paper reports the results of a study of soil factors which affect the uptake of cesium and its possible control.

Methods

The naturally occurring exchangeable potassium content of the four soils chosen for this study varied from 0.092 to 0.376 meq. per 100 grams of soil. They were collected from the plow layer of tilled soils on four midwestern farms. The soils were mixed with cesium-137 at a level of 1000 c.p.m. per gram. About 5 kg. of soil were dispensed into polyethylene containers. Each soil was planted in triplicate to lettuce, grass, and alfalfa. The containers were placed in steel trays in a greenhouse. All water was applied from the bottom, except for that applied during the germination period when the soil was watered from the top with a hand sprinkler.

Four cuttings of each crop were made in the 1958 growing season. The cuttings were weighed, dried, and ashed at 400° F. for 8 hours. The cut weight per pot of each crop varied from 48 to 96 grams for lettuce, from 15 to 40 grams for grass, and from 23 to 50 grams for alfalfa. Plant ash was digested in 6*N* nitric acid. Radioassay for cesium-137 was performed on the supernatant liquid after oxalate precipitation of calcium. Exchangeable soil cations were determined on the extracted portion after the soil had been leached with 1*N* ammonium acetate. Ion exchange capacities were determined by the method of Frysinger and Thomas (*4*) and do not represent the sum of the exchangeable cations as determined by leaching with ammonium acetate.

Results

Results of chemical analyses of soils are shown in Table I. Average concentrations of cesium-137 and total