yielded values, 259 and 256. The theoretical saponification value for the di-ester is 261. Analysis of the saponification liquor yielded *p*-methoxycinnamic acid m. p. 170° and resorcinol m. p. 115°.

An attempt was made to isolate the mono-ester from the alkaline washings obtained from the above experiments.

The alkaline solution was carefully neutralized with 10% HCl and the neutral solution extracted several times with ether in order to remove any unreacted resorcinol along with the mono-ester. When the aqueous solution was acidified a small amount of impure *p*-methoxycinnamic acid was recovered. The ether-soluble fraction was concentrated and the product dried. A few red crystals melting at 135–137° (with decomposition) were obtained. The quantity of this product was too small to permit its characterization.

REFERENCES.

(1) Jour. A. Ph. A., 17, 958 (1928).
(2) M. Bakunin, *Gazz. chim. ital.*, 34, 178 (1902).

THE ADDITION OF STRONG HYDROGEN PEROXIDE IN THE DETERMINATION OF NITROGEN IN ORGANIC COMPOUNDS.*

BY CHARLES F. POE AND BARTLETT T. DEWEY.

INTRODUCTION.

The amount of nitrogen in some organic compounds is determined successfully by the Gunning or Kjeldahl methods. These two methods have advantages over the Dumas method because of the ease of manipulation and the use of less expensive apparatus. However, the distillation methods are subject to certain limitations because the results in the analysis of compounds containing such groups as nitro, nitroso, azo, azoxy, etc., are inconsistent. Many organic compounds also require a very long period of heating in order to liberate all of the nitrogen.

The investigation reported in this communication was undertaken in order to determine accurately the amount of time saved and the accuracy obtainable when thirty per cent hydrogen peroxide was used in the determination of nitrogen. In the last few years, a number of investigators (1–10) have found hydrogen peroxide to be a valuable oxidizing agent in the decomposition of organic substances, because it hastens the process of digestion and cuts down foaming.

EXPERIMENTAL.

The method employed in the investigation reported in this paper was the Gunning modification of the original Kjeldahl method, which is official with the Association of Official Agricultural Chemists (11). Twenty-five hundredths gram of the organic compound was placed in each of two digestion flasks with ten Gm. of nitrogen-free potassium sulphate and twenty cc. of concentrated sulphuric acid. The contents of both flasks were digested over electrically heated plates, all units of which were of the same construction and gave the same amount of heat. During the digestion, one cc. of a 30 per cent solution of hydrogen peroxide was added to one of the flasks at intervals of ten minutes. The contents of the other flasks were allowed to digest without the addition of the hydrogen peroxide. Each sample was heated until the liquid in the flask was light

* Scientific Section, A. Ph. A., Portland meeting, 1935.

straw color. This color, in the case of the amino compounds, was found to indicate the point at which complete conversion of nitrogen into ammonium acid sulphate was effected.

In Table I are given the results of the analysis of a number of organic amino compounds. The study of the above-mentioned table demonstrates that both the Gunning method and the modification making use of strong hydrogen peroxide give results which are very accurate. The addition of hydrogen peroxide in no way affected the accuracy of the analysis, but did shorten considerably the time required for the completion of the determination. The time saved by the use of the strong hydrogen peroxide varied from 37 to 84 per cent—the average saving being 68 per cent.

TABLE I.—DETERMINATION OF NITROGEN IN AMINO COMPOUNDS.

Organic Compounds.	Time in Minutes. Gunning.	Time in Minutes. Plus H_2O_2.	Per Cent Nitrogen. Gunning.	Per Cent Nitrogen. Plus H_2O_2.	Theoretical.	Time Saved in Per Cent.
Acetanilid	89	56	10.26	10.21	10.37	37.1
Acetphenetidin	119	34	7.61	7.63	7.70	71.4
o-Aminobenzoic Acid	60	30	10.15	10.16	10.21	50.0
m-Aminobenzoic Acid	61	32	10.18	10.13	10.21	47.5
p-Aminobenzoic Acid	60	32	10.20	10.16	10.21	46.7
alpha-Amino-*n*-butyric Acid	123	34	13.57	13.54	13.58	72.4
alpha-Aminocaproic Acid	254	51	10.74	10.63	10.68	79.9
alpha-Aminocaprylic Acid	250	48	8.63	8.78	8.79	80.8
alpha-Aminoisobutyric Acid	131	37	13.59	13.58	13.58	71.8
Benzidine	300	78	15.36	15.22	15.21	74.0
p-Bromoacetanilid	140	39	6.45	6.67	6.54	72.2
3-Bromo-4-acetylaminotoluene	210	41	5.94	5.94	6.14	80.5
p-Bromoaniline	120	39	8.05	8.17	8.14	67.5
o-Chloroacetanilid	212	33	8.20	8.19	8.26	84.4
p-Chloroacetanilid	208	38	8.42	8.40	8.26	81.7
p-Chloraniline	230	49	10.85	11.04	10.99	78.7
1,4 - Diaminobutanehydrochloride	165	37	17.38	17.32	17.39	77.6
p - Dimethylaminobenzaldehyde	214	79	9.26	9.19	9.39	63.1
o-Tolylurea	329	89	18.44	18.46	18.65	72.9
p-Tolylurea	240	76	18.48	18.46	18.65	68.3
2,4,6-Tribromoaniline	60	31	4.30	4.31	4.25	48.3

A series of compounds, containing nitrogen in the nitro form, were investigated next. These compounds were analyzed according to the procedure outlined above. The results are presented in Table II. From the data included in this table, it may be observed that there was considerable reduction in the clearing time, but there was little agreement in the percentages of nitrogen obtained. With these nitro compounds, the digestion was stopped when the liquid had attained a light straw color, and the nitrogen was determined in the usual manner. It was thought that prolonged heating after the straw color was obtained might increase materially the percentage of nitrogen found. In no case where the heating was continued as long as one hour were quantitative results obtained. Quantitative recovery of nitrogen was not only impossible, but check results could not be obtained when two determinations were performed under exactly the same conditions. Many investigators have reported that nitro compound cannot be determined successfully by the unmodified Gunning method.

TABLE II.—DETERMINATION OF NITROGEN IN NITRO COMPOUNDS.

	Time in Minutes.		Per Cent Nitrogen.		
	Gunning.	Plus H_2O_2.	Gunning.	Plus H_2O_2.	Theoretical
o-Nitrobenzoic Acid	330	55	8.19	8.23	8.38
	250	30	7.76	7.76	
m-Nitrobenzoic Acid	270	40	7.97	7.75	8.38
	238	46	8.28	8.16	
p-Nitrobenzoic Acid	270	50	7.82	8.13	8.38
	250	33	7.71	7.48	
o-Nitrophenol	220	60	9.43	7.96	10.07
	170	62	9.17	9.06	
m-Nitrophenol	195	75	8.92	9.67	10.07
	160	95	9.01	9.00	
p-Nitrophenol	250	52	8.09	7.81	10.07
	170	63	8.22	8.41	
o-Nitrobenzaldehyde	120	42	9.21	7.20	9.27
	120	40	8.31	7.30	
m-Nitrobenzaldehyde	126	30	9.32	8.99	9.27
	118	32	8.42	9.00	
p-Nitrobenzaldehyde	125	30	9.33	9.08	9.27
	116	28	9.28	9.07	
1,2,3-Nitrotoluidine	150	50	13.29	13.75	18.41
	180	52	14.91	14.00	
1,3,4-Nitrotoluidine	165	48	12.79	13.02	18.41
	180	53	12.67	13.02	
1,2,4-Nitrotoluidine	165	48	15.44	17.00	18.41
	180	52	17.29	17.27	
1,2,5-Nitrotoluidine	150	52	14.56	15.67	18.41
	180	50	18.02	15.01	
1,4,3-Nitrotoluidine	165	49	11.61	12.87	18.41
	180	51	12.32	14.19	
o-Nitrochlorobenzene	140	55	4.29	3.73	8.89
	140	60	5.02	5.02	
m-Nitrochlorobenzene	150	57	3.79	3.09	8.89
	200	55	4.50	3.53	
p-Nitrochlorobenzene	200	51	3.73	3.36	8.89
	150	47	3.41	2.58	

CONCLUSIONS.

1. The percentage nitrogen in compounds containing the nitrogen in form of the amino group is rapidly and accurately determined by use of strong hydrogen peroxide in the Gunning method.

2. The addition of hydrogen peroxide does not make possible the determination of nitrogen in organic compounds containing the nitro group.

REFERENCES.

(1) Bermann, V., and Laufer, L., *Woch. Brau.*, 41, 221 (1924); *C. A.*, 19, 1927 (1925).
(2) Christensen, L. M., and Fulmer, E. J., *Plant Physiol.*, 2, 455 (1927).
(3) Gubarev, E., *Zhurnal exptl. Biol. Med.*, 6, 261 (1927); *C. A.*, 22, 558 (1928).
(4) Heuss, R., *Z. ges. Brauw.*, 6, 44 (1922), *C. A.*, 17, 2093 (1923).
(5) Kleeman, Von, *Z. angew. Chem.*, 34, 625 (1921); *C. A.*, 16, 1196 (1922).
(6) Koch, F. C., and McMeckin, T. L., *J. Am. Chem. Soc.*, 46, 2066 (1924).
(7) Liljevall, S., *Svensk. Kem. Tid.*, 34, 187 (1923); *C. A.*, 17, 1200 (1923).
(8) Lundin, H., and Ellburg, J., *Woch. Brau.*, 46, 133, 147 (1929).
(9) Provvedi, F., *Atti accad. fisiocrit. Siena*, 3, 423 (1928); *C. A.*, 23, 357 (1929).

(10) Saccardi, P., *Biochem. terap. sper.*, 14, 252 (1927); *C. A.*, 22, 364 (1928).
(11) "Official and Tentative Methods of Analysis," *Assoc. Official Agric. Chem.* (1930), 3rd Edition.

STRYCHNINE IV: LETHAL DOSE STUDIES ON CATTLE AND SHEEP.*

JUSTUS C. WARD AND F. E. GARLOUGH.[1]

The earlier papers in this series (1, 2, 3) have presented data on chemical and physiological tests for strychnine, and on masking of strychnine bitterness by certain chemicals. The present discussion deals with the toxicity of strychnine to cattle and sheep.

Literature on the feeding of measured doses of strychnine to domestic animals to determine the lethal doses is very deficient (4). It is because of this fact that the authors are presenting data accumulated under somewhat adverse conditions, since many more animals were involved in their tests than have been reported upon at any one time previously. In these studies 26 cattle and 65 sheep were used.

The obtaining of such a large number of test individuals was made possible through the coöperation of governmental agencies concerned with the administration of the cattle and sheep-buying programs in Idaho and Wyoming during the fall of 1934. The buying program was carried out as a drought relief measure to prevent starvation of a large number of animals on the depleted ranges during the winter. The animals purchased by the Government were graded down to a class that was called "condemned." These "condemned" animals, by the terms of purchase, were to be slaughtered on the owner's property and were to be destroyed. Many of these individuals were organically sound, but were emaciated to the extent that they would not be able to reach a shipping point in condition to be fed back to usable condition. By following the buying crews it was possible for the authors to obtain animals in fairly good condition for their experiments. The tests were always run with the full knowledge and consent of the owner of the "condemned" stock. Normal stock may differ in susceptibility.

Two major observations were made on each animal studied; *first*, the lethal dose; *second*, the rate and manner in which the animal accepted the ground squirrel poison which was the form in which the strychnine was fed. Because of the fact that both cattle and sheep refused the poisoned grain in many cases it was necessary to resort to forced feeding to obtain the lethal dose data needed. Tabulations will show where this forced feeding was used.

The results seem widely variable, so the correlation of dosage trials will prove of value.

Tables I and II indicate that four of eight "condemned" animals died at 15 mg./Kg.; two of three animals at 16 mg./Kg. and all died at doses of 18 mg./Kg. and above. Doses below 12.50 mg./Kg. were only occasionally dangerous, as in the case of a markedly susceptible animal, since only one of nine cows in this dosage range died.

* Scientific Section, A. PH. A., Portland meeting, 1935.
[1] Control Methods Research Laboratory, Bureau of Biological Survey, Denver, Colorado.