THE DETERMINATION OF CYANIDE IN SEEDS

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Previous work[1,2] has shown that cyanides are widely distributed in the plant kingdom and occur mainly, if not entirely, as glycosides from which hydrocyanic acid is liberated by treatment with acid or suitable enzymes. The concentration varies widely in different natural orders, varieties and species, and in different parts of the same plant. It is affected by age, cultivation, and climatic conditions. Calculated as percentage of the cyanide radical, it rarely exceeds 0·2 per cent. of the weight of the plant. A more recent summary[3] gives figures ranging from 0·005 per cent. (Cassava root) to 0·18 per cent. (bitter almonds) or 50 to 1800 parts per million. The present work is concerned mainly with concentrations below 100 p.p.m. At these low levels toxicity is probably not an important factor, and will therefore not be discussed here.

Apart from questions of toxicity, cyanides may exert several important functions in plants. They may control enzyme actions (e.g., inhibit ascorbic acid oxidase and thus stabilise vitamin C), or take part in the phytosynthesis of nitrogenous compounds. A recent report[4] that vitamin B_{12} is distinguished from the related vitamins of the B_{12} group solely by the presence of a cyano group, complex-bound to the cobalt atom, attaches some interest to the occurrence of cyanides in seeds. It therefore seemed worth while to explore this subject, using the more sensitive tests now becoming available.

A number of different tests for cyanide were recently reviewed by Feigl[5]. Most of these were not sufficiently sensitive or specific for our purpose. The older silver nitrate-distillation method which is widely employed in toxicology required too large quantities of material to be applied to most of our samples, but was applied to a few of those containing the higher concentrations of cyanide as a check on the more sensitive method we have employed. This is a modification of the pyridine-pyrazolone method introduced by Gehauf and co-workers[6], and developed by Epstein[7] and by Boxer and Rickards[8]. It consists of three stages: (a) chlorination of cyanide with chloramine T to produce cyanogen chloride. (b) formation of glutaconic aldehyde by treatment of the cyanogen chloride with pyridine to give cyanopyridinium chloride, which hydrolyses to glutaconic aldehyde. (c) development of a blue colour by treatment of the glutaconic aldehyde with 1-phenyl-3-methyl-5-pyrazolone, yielding a blue dye which is stabilised by the presence of a trace of bis-pyrazolone in the reagent.

Experimental

Commercial 1-phenyl-3-methyl-5-pyrazolone was recrystallised from 95 per cent. ethanol, giving brownish pink prisms, m.pt. 127°C.

Commercial pyridine was redistilled (b.pt. 114° to 115°C.).

Commercial phenylhydrazine was redistilled giving golden yellow crystals, m.pt. 19·5°C.

The bis-pyrazolone was prepared by Knorr's method, using an excess of phenylhydrazine. The recrystallised 1-phenyl-3-methyl-5-pyrazolone was dissolved in 95 per cent. ethanol and refluxed for 5 hours with freshly distilled phenylhydrazine. The insoluble bis-pyrazolone was filtered hot, well washed with 95 per cent. ethanol and dried. The melting-point of successive crops, consisting of colourless, diamond-shaped prisms, was above 320°C. (decomp.).

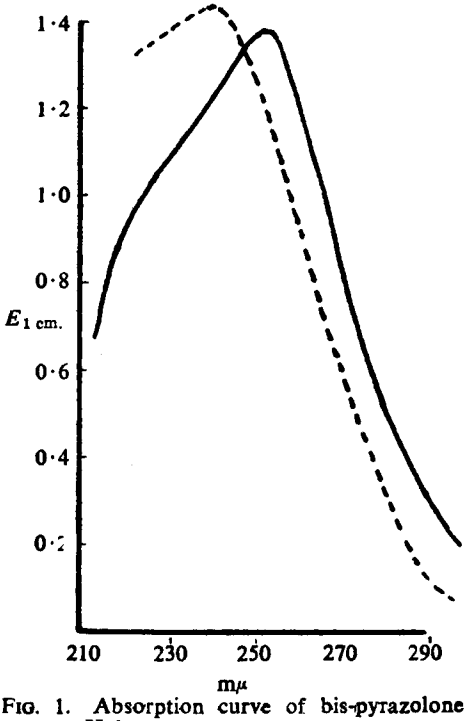

FIG. 1. Absorption curve of bis-pyrazolone at *p*H 1 - - - - - -
at *p*H 12 ———.

The first yields were poor, but could be greatly improved by taking off successive crops of bis compound by concentrating *in vacuo* and standing in the refrigerator overnight. The solubility of the bis compound in water was very slight. The solubilities in 0·4 N sodium hydroxide or 2 per cent. hydrochloric acid were sufficient for examination on the Unicam spectrophotometer, which showed a well marked absorption curve, approximate max. 241 mμ at *p*H 1, shifting to 253 mμ at *p*H 12 (see Fig. 1). The absorption curve was sufficiently steep to permit clear differentiation from phenylhydrazine (max. *ca* 310 mμ in water) and from uncondensed pyrazolone (max. *ca* 254 mμ in water), both of which were possible contaminants.

The pyridine-pyrazolone reagent, made fresh each day according to the directions of Epstein, was stored in amber bottles, under refrigeration and discarded whenever a pink colour developed.

Spectrophotometric characterisation of the blue colour. When examining the blue colour given by the cyanide, Epstein used the Coleman Universal spectrophotometer No. 11 with PC-4 filter in front of the photocell, thus measuring the extinction at about 630 mμ. Boxer and Rickards used a Coleman Model 14 spectrophotometer with PC-5 filter, measuring the extinction at about 620 mμ. As none of these workers had published the absorption curve of the blue colour, we thought it desirable to determine this (see Fig. 2). Our data showed the maximum to lie between 628 and 630 mμ, with a broad peak but fairly steep fall on either side.

The ratio of readings at the maximum to those at 642 mμ and at 602 and 582 mμ, enabled us readily to check the identity of cyanide obtained from different sources, and to show the absence of interfering colours, which occur mainly at wavelengths below 628 mμ.

Chlorination conditions. Boxer and Rickards introduced chlorination at 0°C., which avoided variations in the final colour due to use of different chlorination temperatures. They also stabilised the chlorination *p*H by strongly buffering with potassium dihydrogen phosphate—added to the chloramine T solution just before use. This, however, gives a colloidal suspension, from which the chloramine T may separate within a few minutes as a cloud, or even in clumps. As this was seriously affecting our spectrophotometer readings, we tried to overcome the difficulty by the use of other buffers or even by simple addition of dilute sulphuric acid to bring the *p*H within the range of 6 to 8 recommended by Boxer and Rickards. Our results were still variable. We then determined the effect of the coloration *p*H on the final colour intensity, and found that it was necessary to control this *p*H still more closely in order to obtain consistent results (see Fig. 3). This we did by reverting to the chloramine T-phosphate reagent of Boxer and Rickards, but storing this at 0°C. which satisfactorily hindered cloud formation.

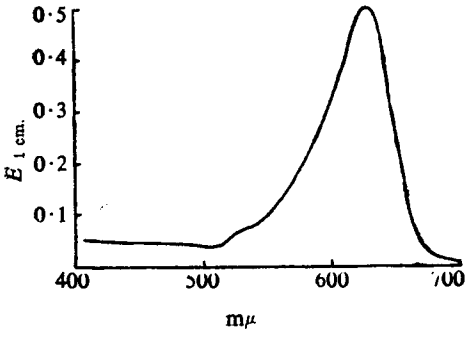

FIG. 2. Absorption curve of blue colour given by CN' with pyridine pyrazolone reagent.

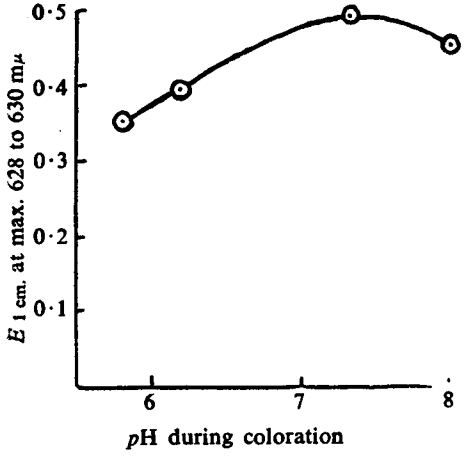

FIG. 3. Effect of *p*H during coloration on intensity of blue colour (measured spectrophotometrically at max. 628 to 630 mμ) produced by pyridine-pyrazolone reagent on 1μg. of cyanide.

The percentage of chloramine T in Boxer and Rickards' reagent is 0·0625, as compared with 1·0 in the reagent used by Epstein. We found that the use of higher concentrations of chloramine T caused the subsequent colour to develop more rapidly, but it was also less stable because of bleaching.

Therefore we have adopted the lower concentration used by Boxer and Rickards, and found it satisfactory.

Rate of development and fading of colour. The rate of development of the colour is most rapid during the first few minutes, when it may quickly reach half of the maximum intensity working at a normal laboratory temperature of 15° to 20°C. This rate steadily diminishes, so that an hour may be needed to reach 90 per cent. of the maximum intensity, and 2 or 3 hours to reach the final maximum. We confirmed Boxer and Rickards' finding that the colour develops more rapidly at higher temperatures, but found that these can also cause it to fade more rapidly. We therefore prefer not to use temperatures higher than 37°C., and have obtained quite satisfactory results at room temperature. Although the visual appearance of the colour alters gradually from an initial pink to a final blue, measurements at the different wavelengths quoted above show constant ratios, showing that the rate of development is the same at these different wavelengths (see Fig. 4). This enables the presence of interfering substances to be detected within a few minutes. After the maximum intensity has been reached, readings at the different wavelengths remain constant for a short time, and then fall gradually, though at a slower rate than that at which the colour developed. Part of this apparent decrease in colour intensity may, however, be due to a slow increase in the colour of the blank, against which all the readings are taken. Usually the colour of the blank is less than 2 per cent. of that given by a standard CN′ solution containing 0·5 p.p.m. As the reagent ages the magnitude of the blank increases.

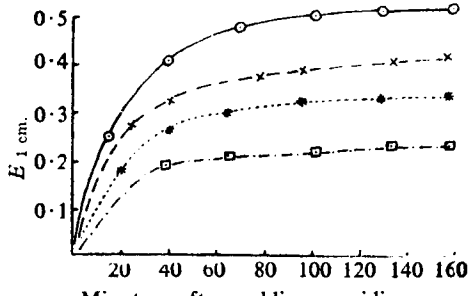

FIG. 4. Rate of development of blue colour given by pyridine-pyrazolone reagent on 1 μg. of CN′ as measured by absorption at different wavelengths

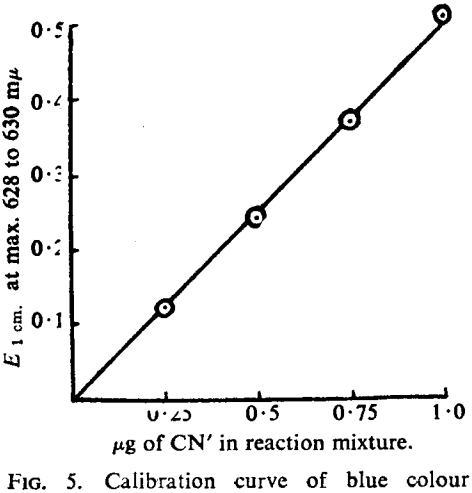

FIG. 5. Calibration curve of blue colour (measured spectrophotometrically) at max. 628 to 630 mμ) produced by pyridine-pyrazolone reagent on different quantities of cyanide in reaction mixture of 8·4 ml.

Calibration data. A number of calibration experiments were carried out with solutions of potassium cyanide A.R. in 0·1 N sodium hydroxide. freshly diluted from a stock solution containing 100 p.p.m. of CN′ which had been titrated against 0·02 N silver nitrate. 0·4 ml. of the clear chloramine phosphate reagent of Boxer and Rickards was mixed with 2 ml. of the standard cyanide solution in a stoppered test tube and put in an iced water-bath for 2 to 3 minutes. The tube was then removed to a beaker of water at 37°C. (or at room temperature) and 6 ml. of Boxer and Rickards' pyridine-pyrazolone reagent added and the time noted. Some of the reaction mixture was put in a cuvette and the extinctions at the above wavelengths were determined on the Unicam spectrophotometer against a blank prepared at the same time, using 2 ml. of 0·1 N sodium hydroxide instead of 2 ml. of standard cyanide solution. Readings were taken at intervals until the maximal extinctions were obtained. Typical results, summarised in Figure 5, support Epstein in showing good linearity of response. As little as 0·25 μg. of CN′ can be determined with a probable error of 2 or 3 per cent. When working with the lower concentrations of cyanide we found it advisable to use saliva traps on all our pipettes. Saliva may contain sufficient thiocyanate to produce the same blue colour as is given by cyanide under the above conditions (see Table I). Thiocyanate occurs in many biological fluids, including milk, as well as in water, in amounts which are quite non-toxic and merely indicate normal functioning of essential biological processes.

TABLE I

SPECTROPHOTOMETRIC DATA ON BLUE COLOURS GIVEN BY PYRIDINE-PYRAZOLONE REAGENT ON CYANIDES AND THIOCYANATES OBTAINED FROM DIFFERENT SOURCES

Source of blue colour	Extinctions at 582, 602 and 642 mμ as percentage of extinction at maximum		
	582mμ	602mμ	642mμ
Cyanides			
Potassium cyanide A.R.	45	66	77
Bitter almonds A	44	66	77
„ „ D	44	65	74
Linseed A	44	66	79
„ D	44	66	76
Phaseolus lunatus	45	66	79
Rubber	46	67	80
Thiocyanates			
Ammonium thiocyanate A.R....	43	62	90
Saliva	43	67	83
Milk	59	68	93

A = aerate. D = distillate.

NOTES:—

The figures are means of all results obtained on different samples. In saliva the thiocyanate content of samples from different subjects ranged from 1 p.p.m. (non smoker) to 50 p.p.m. (smoker). In milk the thiocyanate content ranged from 1 p.p.m. to 6 p.p.m. in samples from different cows. The 642 mμ extinctions for thiocyanates are on the whole higher than those for cyanides. More evidence will be needed to determine whether this difference is significant.

The term " aerate " is used to describe the 0·1 N sodium hydroxide solution containing the hydrocyanic acid which was driven over into it by the aeration process:

Determination of cyanide in plant materials. The modified pyridine-pyrazolone method was employed to determine cyanide in the plant materials (mostly seeds) listed in Table II. They were rapidly powdered, with precautions to avoid loss of cyanide. Weighed amounts were suspended in glass-distilled water in a reaction vessel containing 2 to 3 g. of tartaric acid. The hydrocyanic acid thus liberated was driven off by a

TABLE II

SEEDS AND OTHER MATERIAL EXAMINED

Year	Source	Common name	Botanical name
1950	SP	Almonds, bitter	*Prunus communis*, Arcang var. Amara, Scheider
		,, ,, Italian	,,
		,, ,, Morocco	,,
1949		Beans, broad, Carters Claudia aquadulee	*Vicia faba*
1949		Beans, French, Carters Lightning	*Phaseolus vulgaris*, L.
1949		Beans, runner, Carters Streamline	*Phaseolus multiflorus* Willd.
1920	II	Beans, runner (Burma)	*Phaseolus vulgaris* L.
1950	M	Beans, soya, black	*Glycine hispida* Max.
1950	M	,, ,, white	*Glycine hispida* Max.
1950	M	,, ,, white	
1950	M	Bengal gram	*Cicer arietinum* var. L.
1950	M	Black gram	*Phaseolus mungo* L.
		Buckwheat	*Fagopyrum esculentum Moench.*
1936	II	Castor, Ceylon A	*Ricinus communis*
1936	II	,, ,, B	,,
1937	II	,, Hadramant	,,
1931	II	,, Seychelles	,,
1931	II	,, Sudan	,,
1923/6	II	,, Tanganyika	,,
	SP	,,	,,
1934	II	Cattle melon	*Citrullus vulgaris*
	II	Food gram (N. Nigeria)	*Hibiscus sabdariffa* L.
1936	II	Granadilla (Kenya)	*Passiflora quadrangularis* L.
1950	SA	Guava, fruit	*Psidium guajava* L.
1950	SA	,, seeds	,,
1950	M	Horse gram	*Dolichos biflor* L.
1938	II	*Jatropha multifida*	
1950	M	Kachang puteh	*Cicer arietinum*
1925	II	Kaffir corn, var. Jebere (Tanganyika)	
	II	Kaffir corn, red	
1919	II	Lima bean, var. Bosape (Burma)	*Phaseolus lunatus* L.
1919	II	Lima bean, var. Pelukpya (Burma)	,,
1949	ODF	Linseed	*Linum usitatissimum*
	SP	,,	,,
1950	NIAB	Lupin, yellow	*Lupinus luteus*
1949	ODF	Maize, var. White Horse Tooth	*Zea mays* L.
	II	Millet, Nigeria	*Pennisetum glaucum*
1950		,, Turkish	
1950	M	Mung bean	*Phaseolus aureus*
1950		Padi, Italian	*Oryza sativa* L.
1950	M	Peas, black	
1950	M	Peas, garden (Foremost)	*Pisum sativum* L.
	SP	Quince	*Pyrus cydonia*
1913	II	Ragi (Singapore)	*Eleusine coracana* Gaertn.
1950	M	Ragi (Zanzibar)	
1950	M	Red peas (rice beans)	*Phaseolus calcaratus* Roxb.
		Rubber, Malaya	*Hevea brasiliensis*
1913	II	,, Uganda	*Hevea ceara*
	II	,, Zanzibar	
1950		Vetch, wild	*Vicia sativa*
	SP	Wild cherry bark	*Prunus serotina* Ehrh.
	II	Wild croton (Ceylon)	*Jatropha gossypifolia*

NOTES :—
II = Imperial Institute, London.
M = University of Malaya, Singapore.
NIAB = National Institute of Agricultural Botany, Cambridge.
ODF = Ovaltine Dairy Farm, King's Langley, Herts.
SP = School of Pharmacy, University of London.
Year = Year of crop, except for M samples, where it represents year purchased in market.
All materials in this Table are seeds unless otherwise stated.

stream of nitrogen which had been passed through the 3 traps of Boxer and Rickards, containing acid ceric sulphate, acid silver sulphate and

0·1 N sodium hydroxide respectively. The hydrocyanic acid was driven through 3 further traps containing acid ceric sulphate, acid silver sulphate and 0·1 N sulphuric acid respectively, and absorbed in 5 ml. of 0·1 N sodium hydroxide. A second trap of 0·1 N sodium hydroxide followed to check that absorption of hydrocyanic acid was complete in the first trap. By using up to 50 g. of the plant material in the reaction vessel, and collecting the hydrocyanic acid in 5 ml. of 0·1 N sodium hydroxide, we were able to determine CN' in concentrations as low as 0·01 μg./g. of plant material, or 0·01 p.p.m. These concentrations are much lower than seem to have been explored previously, though they are still much too high for the determination of cyanide liberated by illumination of any such materials which may contain vitamin B_{12}, a question we have not investigated in the present work.

The above apparatus was constructed from resistance glass boiling tubes, 25 × 200 mm., connected by suitable glass and rubber tubing. For the reaction vessels we used wide-mouthed bottles of different sizes. These were less than half full and with nitrogen passing through at 500 to 1000 ml. per minute, very little liquid was carried over by frothing. Any liquid thus carried over was caught in an empty trap, so that none of it reached the other traps. Blank and recovery experiments showed that, under our experimental conditions, the apparatus was not yielding any interfering substances which could either inhibit or produce the blue chromogen.

The *p*H in the reaction vessels was always well below 5, usually between 3 and 4, thus preventing oxidation to cyanide of any thiocyanate present. In view of these precautions, and our spectroscopic characterisation of the blue colours, we believe that these were all due to cyanide derived from the plant materials.

Comparison of pyridine-pyrazolone method with silver nitrate-distillation method. Whilst the silver nitrate-distillation method was not sufficiently sensitive to determine the low concentrations of cyanide we have detected in different samples, we thought it desirable to compare this method with the pyridine-pyrazolone method on samples containing high concentrations of cyanide. The two methods were therefore applied to a sample of bitter almonds, which had been freshly powdered with precautions to avoid loss of cyanide.

For the silver nitrate-distillation method 5 g. was placed in the distillation flask with 200 ml. of distilled water and about 1 g. of tartaric acid. Steam was then passed into the heated flask and the distillate collected in 20 ml. of a solution containing 0·5 g. of potassium hydroxide in the receiver. Not less than 150 ml. of distillate was collected. This was made up to 250 ml. and aliquots titrated with 0·02 N silver nitrate.

For the pyridine pyrazolone aeration method 0·5 g. of the sample was placed in the reaction vessel with about 5 g. of tartaric acid and 50 ml. of distilled water. The hydrocyanic acid liberated was carried by a stream of nitrogen through the traps and collected in 5 ml. of 0·1 N sodium hydroxide. After at least 1½ hours' aeration the 0·1 N sodium hydroxide in the collecting vessel was suitably diluted and aliquots taken for determination of cyanide by the pyridine-pyrazolone method.

On this sample of bitter almonds our first experiments showed good agreement between the results given by the two methods, the silver nitrate-distillation method indicating 994 p.p.m., and the pyridine-pyrazolone aeration method indicating 975 p.p.m. (see Table III). When

TABLE III

DETERMINATION OF CYANIDE IN BITTER ALMOND SAMPLES BY THE SILVER NITRATE AND PYRIDINE-PYRAZOLONE METHODS (ALL RESULTS AS P.P.M. OF CN')

Methods	Samples		
	School of Pharmacy	Italian	Morocco
Pyridine-pyrazolone			
(*a*) on aerates			
(i) using tartaric	975*	138	155
	98	170	65
(ii) after steeping in water, without tartaric acid		12	14
		13	36
(*b*) on distillates	790	1640	725
	976		
Silver nitrate on distillate	857	1997	915
	994		
Means of results on distillates	904	1819	820
Means of results on aerates using tartaric acid, without steeping (excluding *)	98	154	110
Aerate results as percentage of distillate results ...	11	8	13

the pyridine-pyrazolone method was applied to the distillate it indicated 976 p.p.m. However, when the assays were repeated on another portion of the sample, the pyridine-pyrazolone aeration method gave a much lower result of 98 p.p.m., or only about 10 per cent. of the previous figures. A further distillation on this sample gave 857 p.p.m. when titrated with silver nitrate, and 790 p.p.m. when tested by the pyridine-pyrazolone method. We did not think that the low result of the aeration method was due to unevenness of the sample, which had been thoroughly mixed and had given consistent results in all the other experiments. As no more of this batch of bitter almonds was immediately available we obtained two further samples of Italian and Morocco bitter almonds respectively, and repeated our experiments on these. The Italian bitter almonds gave low results by the aeration method, i.e., 138 and 170 p.p.m. in duplicate experiments. A distillation experiment on these almonds gave 1997 p.p.m. when titrated with silver nitrate, and 1640 p.p.m. when treated with the pyridine-pyrazolone reagent. The Morocco almonds gave by aeration 155 and 65 p.p.m. in duplicate experiments, as compared with 915 p.p.m. by silver nitrate on the distillate, and 725 p.p.m. by the pyridine-pyrazolone reagent on the distillate.

The results of all these experiments seem to show that the aeration method as described above, could not be relied upon to give satisfactory results in the estimation of cyanide in seeds. Turning back to the work carried out by Dunstan and Henry[9] 50 years ago on the determination of cyanide in seeds and other plant materials, we found that they liberated

the cyanide from materials by maceration in water at room temperature for a few hours and distillation without any addition of tartaric acid. Thinking that the tartaric acid we used in our aeration experiments might have interfered with the breakdown of the cyanogenetic glycosides by the natural enzymes, we then carried out some experiments in which the almonds were soaked in distilled water for 2 days at room temperature in a closed apparatus before being aerated in it without addition of tartaric acid. The results obtained by the pyridine-pyrazolone method were remarkably low, i.e., 12 and 13 p.p.m. in duplicate experiments on the Italian almonds, and 14 and 36 p.p.m. in duplicate experiments on the Morocco almonds. When we took the residues from these aerations and subjected them to steam distillation, considerable amounts of cyanide were obtained, i.e., 333 and 395 p.p.m. for the Italian almonds, and 869 for the Morocco almonds, showing that considerable quantities had not been liberated during the maceration process with water alone. In the case of the Morocco almonds the sum total of 25 p.p.m. of cyanide (average) by aeration plus 869 by distillation of the residue was 894 p.p.m., similar to the average figure of 820 p.p.m. found by direct distillation (see Table III). In the case of the Italian almonds the sum total of 12·5 p.p.m. cyanide (average) by aeration plus 364 p.p.m. (average) by distillation of the residue was 376·5 p.p.m. This result is very much lower than that obtained by direct distillation (1819 p.p.m. average), a loss for which we have not yet a satisfactory explanation. A final attempt to increase the liberation of cyanide was made by carrying out the aeration at about 90°C. This also proved ineffective. We therefore concluded that for the estimation of cyanide in bitter almonds the aeration method is not suitable.

Comparison of aeration method with distillation method on linseed. The Ovaltine Dairy Farm sample of linseed gave by the aeration method (using tartaric acid) results of 1 and 2 p.p.m. in duplicate assays. When steeped for 2 days in water at room temperature and then aerated with tartaric acid, results of 3·7 and 3·9 p.p.m. were obtained in duplicate experiments. Thus the linseed differed from the bitter almonds in liberating more cyanide when steeped in water to promote enzyme action. However, the amount of cyanide thus liberated was still only a small fraction of the total available, for when the residues from these aeration experiments were steam distilled, the cyanide obtained, as measured by the pyridine-pyrazolone method, was equivalent to 124 p.p.m. The distillation method applied to the same sample of linseed without previous steeping gave a result of 116 p.p.m. An aeration experiment was carried out on linseed to which was added a known amount of potassium cyanide solution. It showed practically complete recovery of the added cyanide, indicating that the aeration apparatus was functioning normally, and that the linseed did not retain the free cyanide. We therefore concluded that the low results given by the aeration experiments were due to incomplete liberation of the bound cyanide under our experimental conditions.

The sample of linseed from the School of Pharmacy also gave low results by the aeration method, when applied direct in presence of tartaric acid or when applied after a day's maceration in distilled water at room temperature. The distillation method when applied to the residues from the aeration experiments again showed the presence of much cyanide which had not been detected by the aeration experiments. Thus the results on this sample confirmed those obtained on the Ovaltine Dairy Farm sample in showing that the aeration method is unsuitable for determining cyanide in linseed.

Comparison of aeration method with distillation method on other seeds. Although the aeration method had proved unsatisfactory for the determination of cyanide in almonds and linseed, we thought that it should

TABLE IV

CYANIDE CONTENT (AS P.P.M. OF CN′) OF SEEDS AS DETERMINED BY DISTILLATION AND BY AERATION METHODS

Methods	Distillation using tartaric acid	Aeration	
		Using tartaric acid	*After steeping (without tartaric acid)
Almonds, bitter, Italian	1819	154	13
„ „ SP	904	98	
„ „ Morocco	820	110	25
Linseed, ODF	124	1·5	4
„ SP	32	3	2·5
Rubber (Malaya)	54	1	0·4
Cattle melon	41	2	
Wild cherry bark	38	1	
Phaseolus lunatus, var. Pelu	20	20	
„ „ var. Bosape	17	14	
Quince	13	3·5	
Phaseolus vulgaris, var. Bosape	8	8	
Buckwheat	7	7	
Castor, Hadramant	7	nil	
Vetch	5	1	
French beans	—	4	1·8
Lupin, yellow	2	1·5	1
Broad beans	—	1	
Runner beans	2	0·16	0·04
Rubber (Ceara)	—	3	
Guava	3	3	
Bengal gram	3	0·03	
Peas, Foremost	0·5	nil	

NOTES:—

* The residues from these aeration experiments were distilled, after addition of tartaric acid to bring the pH below 3. Determination of cyanides in the distillates usually gave total cyanide contents similar to those obtained on the original material by distillation using tartaric acid.

Cyanide was not detected in any of the seeds in Table II which are not included in the above Table.

be tried on a number of other seeds before discarding it in favour of the distillation method which is much more difficult to apply to the low concentrations of cyanide in which we are interested. The mean results of duplicate or triplicate determinations by both methods are given in Table IV. It will be seen that, apart from buckwheat, *Phaseolus* and guava the distillation method always gave higher results up to about 100 times the results given by the aeration method. We therefore consider the distillation method to be more reliable for the determination of cyanides in seeds and other plant material, and suggest the following modifications which greatly improve its specificity and sensitivity. (a) Use of the pyridine-pyrazolone method in place of titration with silver nitrate to measure the cyanide in the distillate. (b) If the concentration of cyanide in the distillate is too low for accurate measurement, the distillate should be put in the aeration apparatus, tartaric acid added to bring the *p*H below 3, and the liberated hydrocyanic acid carried over and collected in 0·1 N sodium hydroxide. In this manner the concentration of cyanide can be increased several hundred fold.

Dealing first with seeds in which the presence of cyanide was to be expected from the findings of previous workers, the results on the two *Phaseolus lunatus* samples are of interest in view of the fact that when examined in the Imperial Institute over 30 years ago they were found to contain 450 and 500 p.p.m. of cyanide. Some of the cyanogenetic glycosides in seeds can persist after prolonged storage in a herbarium. The content of about 100 p.p.m. of cyanide found in the Ovaltine Dairy Farm sample of linseed is lower than the contents of 200 to 380 p.p.m. quoted by Thorpe[3]. Possibly this was due to difference in variety. This sample of linseed had a high degree of viability, as shown by the 93 per cent. germination which we obtained. The occurrence of cyanide in some varieties of rubber seeds is well known, and our findings on two different samples are not surprising. No cyanide was found in the Zanzibar rubber seeds, which were older and would not germinate in our hands.

Turning to seeds in which the presence of cyanide does not appear to have been previously reported, our finding of 33 p.p.m. in cattle melon seed is of interest in view of their use in cattle feeding. The remaining seeds mentioned in Table V are all used in human or animal nutrition. The question arises as to the significance of the small amounts of cyanide found in them, and whether under certain conditions these amounts might not be considerably increased.

SUMMARY

1. The pyridine-pyrazolone aeration method of Epstein and of Boxer and Rickards for determining cyanide has been applied to a variety of seeds.

2. On 3 different samples of bitter almonds it gave much lower results than the silver nitrate distillation method as used in toxicology.

3. The low results by the aeration method appeared to be due to failure to liberate cyanide from the cyanogenetic glycosides present.

4. The pyridine-pyrazolone colour test gave much better agreement with the silver nitrate titration on distillates from the bitter almonds. It was, therefore, used to determine cyanide in distillates from a number of different kinds of seed in which the concentration was too low for measurement by titration with silver nitrate, and on most of these gave much higher results than were given by the aeration method.

5. It is, therefore, suggested that cyanide in seeds and other plant materials should be determined by the distillation method. The specificity and sensitivity can be greatly increased by applying to the distillates the pyridine-pyrazolone colour test with the spectrophotometric modifications described. If the concentration of cyanide in the distillates is too low, it can be increased several hundred fold by aeration, and by applying the colour test to the aerates, thus enabling as little as 0·01 p.p.m. of cyanide to be determined.

6. By these means cyanide has been determined in different seeds in which its presence had not previously been reported.

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REFERENCES

1. Gresholf—quoted in article on Cyanogenesis in Plants, *Bull. Imp. Inst.*, 1906. **4**, 329.
2. Robinson, *Biol. Rev. and Biol. Proc. Cambridge Phil. Soc.*, 1931, **5**, 126.
3. Thorpe's *Dict. Applied Chem.*, 4th edition, Vol. III, p. 499.
4. Brink, Kuehl and Folkers, *Science*, 1950, **112**, 354.
5. Feigl, *Qual. Analysis by Spot Tests*, Elsevier Publishing Co., N. York-Amsterdam, 1946.
6. Gehauf, Witten and Falkof, personal communication to Epstein, June, 1944.
7. Epstein, *Anal. Chem.*, 1947, **19**, 272.
8. Boxer and Rickards, *Arch. Biochem.*, 1951, **30**, 372.
9. Dunstan and Henry, *Brit. Ass. Rep.* York, 1906.

DISCUSSION

This paper was presented by DR. S. G. WILLIMOTT.

The CHAIRMAN asked whether the cyanide content was intended to be a standard of purity for a particular source of seed.

MR. H. DEANE (Sudbury) said that the formation of benzaldehyde and hydrocyanic acid was not a simple chemical reaction, and that a time factor was involved such that yields of hydrocyanic acid fell with long maceration.

DR. J. W. FAIRBAIRN (London) explained that his interest was not so much in the technique but in the work which must obviously follow as it would appear that the cyanogenetic glycosides played an important part in the plant physiology. If so, it would be one of the rare instances where substances of therapeutic value were also shown to be of value to the plant. Referring to the assay of two samples of *Phaseolus lunatus* which were first examined 30 years ago, it would appear that the loss was 96 per cent. over that period which was surprisingly heavy.

DR. WILLIMOTT, in reply, stated that methods employed in the past had lacked sensitivity and the method under discussion appeared to offer a highly specific colour reaction. Suitable conditions had been worked out in order that the method might form the basis of further work on plant phytosynthetic processes. Losses on maceration had been encountered, and it was only possible to recover small amounts of cyanide after prolonged maceration. The figures quoted for the samples of *Phaseolus lunatus* were those actually obtained, but he himself was surprised to find that a small amount of cyanogenetic glycoside was still present.