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FACTORS AFFECTING THE EXTRACTABILITY AND MAK CHROMATOGRAPHY OF NUCLEIC ACIDS FROM WOODY PLANT TISSUE

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Abstract—Factors affecting the yield of the different classes of nucleic acids extracted from woody plant tissues were investigated using MAK chromatography. Sodium lauryl sulfate (2·0%) improved the yields of nucleic acids by 187 per cent. Shaking of the homogenate for 30 min increased yields an additional 40 per cent, probably by allowing sodium lauryl sulfate to more effectively dissociate nucleic acids from protein and membranes. In Korean boxwood leaves, the addition of phenol before the liberation of nucleic acids by sodium lauryl sulfate reduced yields of DNA and r-RNA. Lyophilization of tissues permitted the use of dry grinding techniques which improved cell breakage and increased yields of nucleic acids. Nucleic acid degradation during lyophilization, due to the thick tissue cuticle which prevented evaporative cooling, was minimized by maintaining the sample at −20° during drying. Precipitation of nucleic acids from dogwood bark with 2-ethoxyethanol at 0–4° improved MAK separation by eliminating interfering substances which competed with nucleic acids for binding sites on the MAK column. Degradation of precipitated nucleic acids by sodium lauryl sulfate was prevented by removing it with ether prior to precipitation.

INTRODUCTION

MANY developmental processes in woody plants such as cold acclimation, dormancy, and cell elongation and differentiation, are undoubtedly associated with metabolic changes mediated by nucleic acids. Efforts to establish relationships between these processes and nucleic acid metabolism have been hampered by the lack of a method to isolate nucleic acids from woody plants. Although numerous procedures are outlined for the extraction and fractionation of nucleic acids by MAK (methylated albumin on kieselguhr) chromatography for herbaceous plant tissues,[1–4] they are not directly applicable to woody plants. Recently Li and Weiser[5] outlined a method for nucleic acid extraction from apple twigs, but it did not give high yields especially in the r-RNA (ribosomal ribonucleic acid) fractions. Cells of woody plant species are very resistant to cell breakage, and homogenization techniques which effectively break such cells often mechanically degrade nucleic acids. Incomplete cell breakage results, not only in poor yields of nucleic acids, but in preferential breakage of young, soft cells which may result in selective extractions. In addition, many woody plants contain large quantities of substances which interfere with MAK chromatography.

The following report presents methods for obtaining good yields of nucleic acids from woody tissue for MAK chromatography, and describes the effect of SLS (sodium lauryl sulfate), homogenization time, shaking time and lyophilization on nucleic acid extractability. The leaves of Korean boxwood (*Buxus microphylla* var. *koreana*) and the bark of red-osier

[1] H. L. SANGER and C. A. KNIGHT, *Biochem. Biophys. Res. Commun.* **13,** 455 (1963).
[2] W. BENJAMIN and A. GELHORN, *J. Lipid Res.* **7,** 285 (1966).
[3] R. E. CLICK and D. P. HACKETT, *Biochem. Biophys. Acta.* **129,** 74 (1966).
[4] J. H. CHERRY and H. CHROBOCZEK. *Phytochem.* **5,** 411 (1966).
[5] P. H. LI and C. J. WEISER, *Plant Cell Physiol.* **10,** 21 (1969).

dogwood (*Cornus stolonifera* Michx.) were examined because both are of interest in our research on cold acclimation and neither can be studied successfully using extraction techniques which have been developed for herbaceous plant tissues.

RESULTS AND DISCUSSION

The buffer mixture used in these studies consisted of 0·01 M Tris, 0·15 M NaCl, 0·015 M sodium citrate, 0·01 M 2-mercapthoethanol, pH 7·7 (1 × SSC). Preliminary studies indicated that this buffer system was superior to the more commonly used 0·01 M Tris, 0·06 M KCl, 0·01 M $MgCl_2$, pH 7·6 buffer system for nucleic acid extraction.[5] This is probably due in part to the precipitation of SLS by potassium and magnesium ions during extraction.[6] Lower yields of RNA from plant tissues have been associated with the presence of magnesium ions in the extraction buffer.[7] Also, magnesium has been shown to stimulate ribonuclease (RNase) activity.[8] Phenolic browning of aqueous extracts was prevented by 2-mercaptoethanol (0·01 M). Sodium chloride (0·15 M) was also added to the extraction buffer since it has been shown to disrupt electrovalent linkages between nucleic acids and proteins without affecting the nucleic acids.[9]

As reported by Li and Weiser[5] for apple twigs, bentonite, a nuclease inhibitor, did not aid in the extraction of nucleic acids and therefore was not used. Perhaps SLS, which is also a powerful nuclease inhibitor,[10] may have inhibited the nucleases.

When phenol was used in the first deproteinization step, inconsistent and low yields of nucleic acids were obtained from fresh boxwood tissue preparations. For this reason, a mixture of chloroform–isoamyl alcohol (24:1, v/v) was used in the initial deproteination step. Buffer saturated 88 % liquefied phenol was used in all subsequent deproteination steps.

TABLE 1. THE INFLUENCE OF HOMOGENIZATION TIME ON THE YIELD OF NUCLEIC ACIDS FROM FRESH BOXWOOD LEAVES

	Homogenization time (min)					
Nucleic acids (OD_{260}/8 g fr. wt.)	2	3	4	5	6	7
4s-RNA	1·28	1·30	1·40	1·60	1·45	1·05
5s-RNA	0·73	0·88	0·95	1·06	0·89	0·57
DNA	2·47	2·89	2 98	3·55	3·07	2 22
r-RNA	9·06	10·22	11·26	12·53	10·79	6·08
RNA	11·07	12·40	13·61	15·19	13·13	7·70
Total	13 54	15 29	16·59	18 74	16 20	9·92

8 g (fr. wt.) of boxwood leaves in 40 ml of $CHCl_3$–isoamyl alcohol (24:1, v/v) and 40 ml of 1 × SSC buffer pH 7·7 were homogenized in an icebath with a VirTis "45" homogenizer. Following homogenization, SLS was added to a final concentration of 1 % and the mixture was shaken for 15 min at room temp. Samples were then centrifuged and the aqueous solution was collected and extracted with phenol. Nucleic acids were precipitated from the aqueous solutions in 2 vol. of ethanol. The precipitates were collected by centrifugation, washed twice with ethanol, dissolved in 0·05 M phosphate buffer, pH 6·7, and chromatographed on MAK columns. Nucleic acids were eluted with a linear gradient of NaCl from 0 30 M to 1 25 M in 0·05 M sodium phosphate, pH 6·7.

[6] W. K. ROBERTS and J. D. QUINLIVAN, *Biochem.* **8,** 288 (1969).
[7] R. E. CLICK and D. P. HACKETT. *Biochem. Biophys. Acta* **129,** 74 (1966).
[8] D. M. HANSON and J. L. FAIRLEY, *J. Biol. Chem.* **244,** 2440 (1969).
[9] M. L. MIHAILOVIE, S. A. GRUJIC and D. HADZIJEV. *Biochem. Biophys. Acta* **76,** 101 (1963).
[10] H. NOLL and E. STUTZ, in *Methods in Enzymology* (edited by L. GROSSMAN and K. MOLDAVE), Vol, 12, part B, p. 129, Academic Press, New York (1968)

Effects of Homogenization

Five minutes of homogenization at top speed in a VirTis "45" homogenizer gave the best extraction of nucleic acids from fresh boxwood leaves. Longer periods of homogenization resulted in degradation in all fractions, particularly of r-RNA (Table 1). Degradation was probably due to mechanical shearing from homogenization or from excessive foaming. The lower yields associated with homogenization periods of less than 5 min were probably due to incomplete maceration.

Effects of Sodium Lauryl Sulfate

Georgiev[11] and Kirby[12] have shown that addition of dupanol (sodium lauryl sulfate) to the extraction medium increased the yields of both RNA and DNA (deoxyribonucleic acid). Cherry and Chroboczek[13] found that RNA and DNA yields from peanut cotyledons increased as the dupanol concentration was raised from 0·1 to 4%. SLS at 0·5% has been shown to totally deproteinize plant 80-s ribosomes.[14] Also SLS is a very effective protein denaturing agent which inhibits nucleases.[10]

Various concentrations of SLS (0–3·0%, w/v) were tested to determine the optimal level for extracting RNA and DNA from boxwood leaves (Table 2). Two per cent SLS improved the yield of r-RNA and DNA by 187 and 122 per cent respectively, and gave the highest total yield of nucleic acids. The yield of s-RNA (soluble RNA) (4s + 5s) was as good at 1·0 per cent SLS as at higher concentrations. Concentrations of SLS higher than 2·0 per cent reduced yields. This reduction, which was particularly evident in the DNA and r-RNA fractions at SLS levels above 3 per cent, was probably due to excessive foaming during shaking.

TABLE 2. THE INFLUENCE OF SODIUM LAURYL SULFATE CONCENTRATION ON THE YIELD OF NUCLEIC ACIDS FROM FRESH BOXWOOD LEAVES

Nucleic acids (OD_{260}/8 g fr. wt.)	SLS %				
	0	1·0	1·5	2·0	2·5
4s-RNA	0·95	2·00	2·00	2·18	2·05
5s-RNA	0·54	1·24	1·25	1·31	1·25
DNA	1·80	3·81	3·85	4·00	3·37
r-RNA	5·35	13·37	16·16	17·33	17·12
RNA	6·84	16·61	19·41	20·82	20·42
Total	8·64	20·42	23·26	24·82	23·79

Nucleic acids were extracted as in Table 1, except that the SLS concentration was varied as noted.

A relatively high concentration of SLS (2%) was required for optimal nucleic acid yields as opposed to the low concentration of SLS (0·1–1·0%) commonly used for bacteria and herbaceous plants. This would suggest that in woody plants either a large percentage of the r-RNA is membrane bound or that the dissociation of r-RNA and DNA from membranes and/or protein is more resistant to SLS action.

[11] B. P. GEORGIEV, *Biokhimiya* **24**, 472 (1952).
[12] K. S. KIRBY, *Biochem. J.* **66**, 495 (1957).
[13] J. H. CHERRY and H. CHROBOCZEK, *Phytochem.* **5**, 411 (1966).
[14] E. STUTZ and J. R. RAWSON, *Biochem. Biophys. Acta.* **161**, 564 (1968).

Storage of precipitated nucleic acids in ethanol at −20° for more than 60 hr reduced yields, especially in the r-RNA fraction. It was found that this loss could be avoided by washing the medium containing the nucleic acids with diethyl ether twice prior to ethanol precipitation. The addition of various concentrations of SLS to the nucleic acids extracted from boxwood leaves revealed that SLS concentration was related to RNA degradation during storage (Table 3). At 0–4°, the maximum loss in total RNA occurred at SLS concentrations of 1·25% or higher. At a concentration of 2·5% SLS, RNA was reduced 48% at 0–4° and 29% at −20°.

TABLE 3. THE INFLUENCE OF SODIUM LAURYL SULFATE CONCENTRATIONS DURING STORAGE ON THE RECOVERY OF RNA FROM PRECIPITATED NUCLEIC ACID PREPARATIONS FROM BOXWOOD LEAVES

	% of control	
% SLS	0–4°	−20°
0	100	
0 250	96 8	
0·625	69·3	
1·250	51·7	
1·875	51·7	
2·500	51·7	70 6

Nucleic acids were extracted from boxwood leaves (1 g dry wt.) by homogenizing in a medium containing 1 × SSC buffer, 1% SLS and $CHCl_3$–isoamyl alcohol (24:1, v/v). The homogenate was centrifuged and the aqueous solution collected and extracted twice with phenol. The aqueous solution was collected and the nucleic acids precipitated with an equal volume of cold (0–2°) 10% TCA (trichloroacetic acid). After 20 min the precipitate was collected, washed twice with ethanol and once with ether to remove phenol, TCA and SLS. Then the precipitate was dissolved in 1 × SSC buffer, divided into six equal aliquots and the various concentrations of SLS were added. Two volumes of ethanol were added to each aliquot and the mixtures were stored at 0° or −20° for 20 hr. The precipitates were collected, redissolved in 1 × SSC buffer and reprecipitated with TCA, washed with ether and finally dissolved in an equal volume of 1 × SSC buffer and assayed for RNA by the Orcinol method.[15]

The Effect of Shaking Time on Nucleic Acid Extractability

A study was conducted to determine the optimum shaking time for SLS liberation of DNA[10] and RNA[10,15–17] from associated proteins and/or lipids (membranes). As shown in Table 4, the yield of r-RNA and DNA increased as the shaking time was extended from 0 to 30 min. The beneficial effect of a long shaking period was less pronounced for 4s-RNA which is believed to be in a free state in the cytoplasm. SLS will precipitate when added to a buffer at temperatures lower than 10°. When homogenates are shaken at room temperature,

[15] W. MEJBAUM, *Physiol. Chem.* **258**, 117 (1939).
[16] S. PETROVIĆ, A. BECAREVIĆ and J. PETROVIĆ, *Biochem. Biophys. Acta* **95**, 518 (1965).
[17] S. PETROVIĆ, J. PETROVIĆ and M. E. BAYER. *Biochem. Biophys. Acta* **145**, 193 (1967).

TABLE 4. THE INFLUENCE OF SHAKING TIME ON THE EXTRACTABILITY OF NUCLEIC ACIDS FROM BOXWOOD LEAVES

Nucleic acids (OD_{260}/8 g fr. wt.)	Shaking time (min)			
	0	5	15	30
4s-RNA	1·38	1·62	1·63	1·64
5s-RNA	1·00	1·05	1·05	1·10
DNA	2·88	3 28	3·33	3·37
r-RNA	9·78	11·42	13·55	14·77
RNA	12·16	14·09	16·23	17·51
Total	15·04	17·37	19·56	20·88

Nucleic acids were extracted as in Table 1, except the shaking time was varied.

SLS probably becomes more effective as it dissolves as the buffer warms up to room temperature. Perhaps lithium dodecyl sulfate[10] would be a better choice of detergent since it is soluble at temperatures close to 0°.

The Effect of Phenol on the Extractability of Nucleic Acids

Recently, several investigations have revealed that phenol causes a reduction in the yield of nucleic acids. This has not been generally recognized. Petrovic, Becarevic and Petrovic,[16] working with rat liver, reported that a specific RNA from microsomes behaves like nuclear RNA's in that it cannot be completely extracted unless the membrane structure is destroyed with SLS prior to deproteinization with phenol. Noll and Stutz[10] reported that when SLS is used in combination with phenol, there are often selective losses of nucleic acids by entrapment in precipitates of denatured protein, particularly at interfaces during centrifugation. In work with gametophytes, Ingle and Burns[18] also reported that they could extract no DNA and much lower yields of r-RNA with phenol as compared to chloroform.

To establish the effect of phenol on the amount and types of nucleic acid extracted, boxwood leaves were homogenized in the presence and absence of phenol. The total yields and relative amounts of each type of nucleic acid extracted from fresh boxwood leaves are summarized in Table 5. The highest nucleic acid yields were obtained if phenol was added after the 5 min homogenate had been shaken for 10 min in the presence of SLS. SLS was added immediately after homogenization because of foaming problems. If SLS was added after the tissue was homogenized in 1 × SSC and phenol, there was a 72 per cent reduction in yield. In contrast to phenol, the chloroform–isoamyl alcohol mixture could be added at the time of homogenization with little or no loss of nucleic acid yield. When phenol was used at the time of homogenization, the largest losses of nucleic acids occurred in the membrane or protein bound nucleic acid fractions; namely, r-RNA and DNA. Therefore, it appears necessary to liberate r-RNA and DNA from their association with membranes and proteins before deproteinization with phenol.

Phenol had a different effect on the extractability of nucleic acids, especially DNA, from dogwood bark (Table 6). There was a slight loss of r-RNA if phenol was added at the time of homogenization at 0–4°, but not at 20–21°. The addition of phenol at the time of homogenization increased the yield of DNA 87 per cent at 0–4° and 154 per cent at 20–21°.

[18] J. INGLE and R. G. BURNS, *Biochem. J.* **110,** 695 (1968).

TABLE 5. THE EFFECT OF PHENOL ON THE EXTRACTABILITY OF NUCLEIC ACIDS FROM BOXWOOD LEAVES

Extraction method	Nucleic acids (OD_{260}/8 g fr. wt.)					
	4s-RNA	5s-RNA	DNA	r-RNA	RNA	Total
A	2·44	1 52	3·71	18·45	22·41	26 12
B	2 55	1·56	3·76	20 23	24·34	28 10
C	2 53	1 50	3 41	17 56	21·59	25·00
D	2·14	1·35	2 13	2 07	5·56	7·69
E	2 52	1·53	3 76	19·51	23·56	27 32

A. Homogenized at 0–4° for 5 min in 40 ml of 1 × SSC buffer, 2% SLS, and an equal volume of $CHCl_3$–isoamyl alcohol (24:1, v/v). The homogenate was shaken for 30 min and then centrifuged to recover the aqueous phase. Then the aqueous phase was extracted with phenol for 5 min Finally, the nucleic acids were precipitated from the aqueous solutions with cold ethanol (−20°).

B. Same as A except after homogenization, SLS was added to a concentration of 2% and shaken for 10 min. Then an equal volume of $CHCl_3$–isoamyl alcohol was added, and the sample was shaken for an additional 20 min.

C. Homogenized 5 min in 40 ml of 1 × SSC buffer, 2% SLS, and 40 ml of phenol, at 0–4°. This was shaken for 30 min, centrifuged, and the aqueous solution was extracted once with phenol. Nucleic acids were precipitated with cold ethanol (−20°).

D. Same as C, except SLS was added after homogenization.

E. Same as B, except phenol was substituted for $CHCl_3$–isoamyl alcohol

TABLE 6. THE YIELD OF NUCLEIC ACIDS FROM LYOPHOLIZED (a AND c) AND FRESH (b) BOXWOOD LEAVES (A) AND RED-OSIER DOGWOOD BARK (B)

Nucleic acids (OD_{260}/3·5 g dry wt.)	Homogenization at 0–4° (min)			
	2a	3a	4a	5b
A				
4s-RNA	2·32	2 59	3·31	2 22
5s-RNA	1·48	1·61	2·41	1·48
DNA	3 02	3·85	3·91	3 26
r-RNA	15 93	16·74	19·65	16·70
RNA	19·73	20·94	25·37	20·40
Total	22·75	24 79	29 28	23 66

Nucleic acids (OD_{260}/3·5 g dry wt)	Homogenization					
	0–4° (min)			20–21° (min)		
	2a	4a	4c	5b	4a	4c
B						
4s-RNA	3·99	4·07	4·03	1·42	4 01	4·00
5s-RNA	1·56	1 21	1·16	0·62	1·21	1 22
DNA	0·84	1·19	2·23	0 41	1·85	3·03
r-RNA	13·69	15 10	13 50	5 32	15·45	15·44
RNA	19 24	20·38	18 69	7 36	20·67	20·66
Total	20·08	21·57	20 92	7 77	22·52	23·69

a. Lyophilized samples were homogenized at 0–4° in 40 ml of 1 × SSC buffer. SLS was then added to 2·0% prior to shaking for 10 min. Then an equal volume of phenol was added and the sample was shaken an additional 15 min. After centrifugation, the aqueous solutions were collected and extracted once with phenol. Boxwood nucleic acids were precipitated from the aqueous phase with cold ethanol (−20°) and dogwood nucleic acids were precipitated with an equal volume of 2-ethoxyethanol (0–4°).

b. Fresh sample of equivalent dry weight, extracted as in method a.

c. Lyophilized samples were homogenized in 40 ml of 1 × SSC buffer, SLS added to 2% and 40 ml of phenol. This was shaken 25 min, centrifuged and then treated as in method a.

The Effect of Lyophilization on the Extractability of Nucleic Acids

Lyophilization has been shown to be an excellent technique for preserving organisms.[19] Lyophilized tissue can be ground in the dry state and the resulting powder can be stored for long periods of time. Recently, Leary *et al.*[20] and Kato and Tamaoki[21] have shown that lyophilization has no deleterious effect on the functional integrity of ribosomes.

Dogwood bark and boxwood leaves were lyophilized in a deepfreeze at −20°, ground through a 60 mesh screen in a Wiley mill and then ground further in a motorized mortar and pestle until the material would pass through a 149 μm sieve. Nucleic acid extraction from samples prepared in this way was compared with extraction from fresh bark samples which were chopped into small pieces at 0–4° and then homogenized in a VirTis "45" homogenizer. The yield of all classes of nucleic acids from lyophilized samples was much higher than from fresh samples on a dry weight basis (Table 6). Nucleic acid yields from lyophilized samples homogenized for 2 min were comparable to the yields obtained from fresh samples homogenized for 5 min. There is less opportunity for mechanical degradation of nucleic acids as the homogenization time is reduced. Figure 1 shows the MAK elution profiles of nucleic

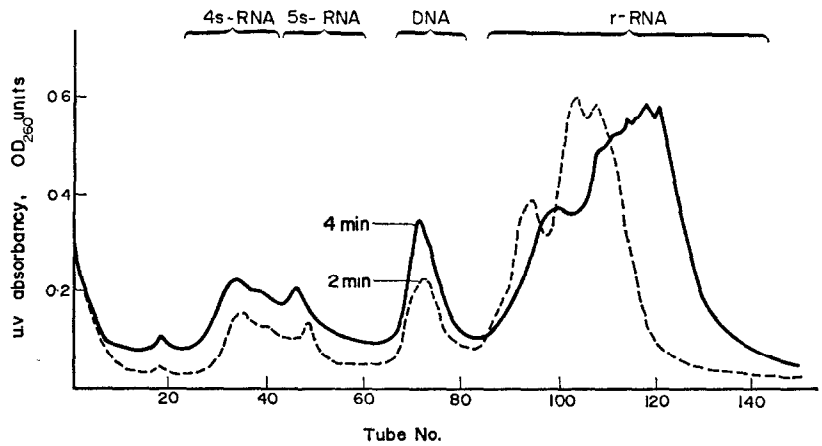


FIG. 1. MAK ELUTION PROFILES OF LYOPHILIZED BOXWOOD LEAVES NUCLEIC ACIDS HOMOGENIZED IN A VIRTIS "45" AT FULL SPEED FOR 2 min (-----), OR FOR 4 min (——).

acids from lyophilized boxwood leaves which were homogenized for either 2 min or 4 min during extraction.

Four minutes of homogenization for lyophilized boxwood leaves gave better over-all yields of nucleic acids than 2 min, but the MAK elution profiles indicated r-RNA degradation. MAK elution profiles of nucleic acids from dogwood bark did not indicate any degradation in samples homogenized up to 4 minutes.

Figure 2 illustrates the importance of lyophilization temperature. As a rule, when samples are lyophilized, they remain frozen due to sublimation cooling. However, the leaves and bark of many woody plants have a thick layer of cuticle which prevents rapid lyophilization, and the frozen samples may thaw during freeze-drying. For example, boxwood

[19] S. M. MARTIN. *Ann. Rev. Microbiol.* **18,** (1964).
[20] J. V. LEARY, A. J. MORRIS and A. H. ELLINGBOE, *Biochem. Biophys. Acta* **182,** 113 (1969).
[21] T. KATO and T. TAMAOKI, *Biochem. Biophys. Acta* **182,** 250 (1969).

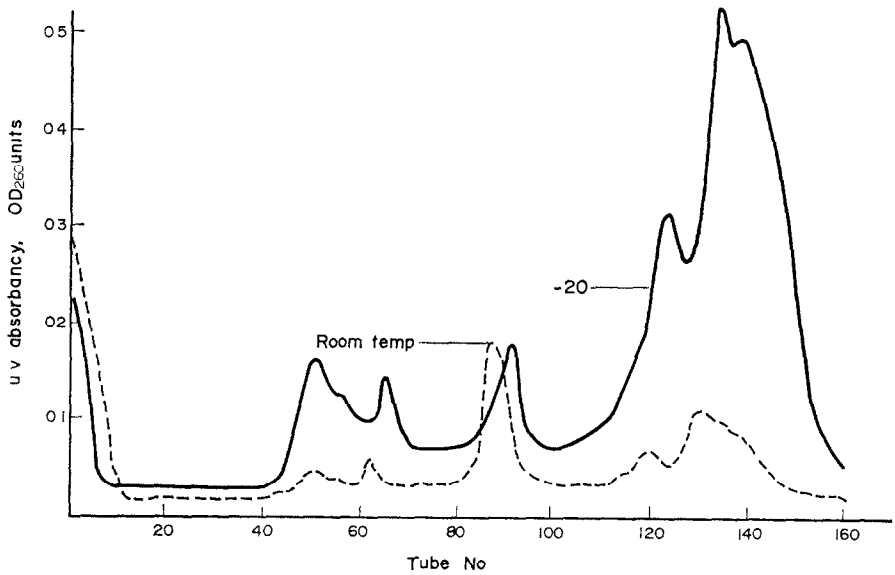


FIG. 2. MAK ELUTION PROFILES OF NUCLEIC ACIDS EXTRACTED FROM BOXWOOD LEAVES LYOPHILIZED at −20° (——) OR AT ROOM TEMPERATURE (-----).

leaves or dogwood bark thaw when lyophilized at room temperature. This results in low yields of nucleic acids and MAK elution profiles are indicative of degradation (Fig. 2). Samples lyophilized in a freezer at −20° gave higher yields, and the MAK elution profiles indicated no signs of degradation. Probably when samples are frozen to be lyophilized, intracellular ice crystallization disrupts compartmentalization, and the nucleic acids are enzymatically degraded upon thawing.

Removal of MAK Interfering Substances

Certain unknown substances from dogwood bark tissue are extracted with the nucleic acids. These substances are precipitated with the nucleic acids in ethanol and interfere with MAK chromatography, probably by competing for binding sites (Fig. 3). As a result, the nucleic acids appear in the 0·2 M NaCl column wash. When the 0·2 M NaCl wash was loaded on an uncharged MAK column, the nucleic acids were absorbed. To avoid this problem, dogwood nucleic acids were precipitated with 2-ethoxyethanol at 0–4°. If the DNA concentration is less than 200 μg/ml, this fraction may not precipitate completely.[22] At higher concentrations, 2-ethoxyethanol completely precipitates all nucleic acid fractions within 5 hr. Lanthanum nitrate or cetyltrimethylammonium bromide to selectively precipitate nucleic acids may also have possibilities.

It was found that boxwood leaf or dogwood bark nucleic acids need not be precipitated, as they can be separated from such low molecular weight compounds as phenol, SLS and pigments by Sephadex G-25 chromatography prior to loading on MAK columns. This permitted a much shorter time from extraction to fractionation which minimizes the chance of enzymatic or chemical degradation (e.g. SLS) of nucleic acids.

[22] D. E. KOHNE, *Biophysical J.* **8**, 1104 (1968).

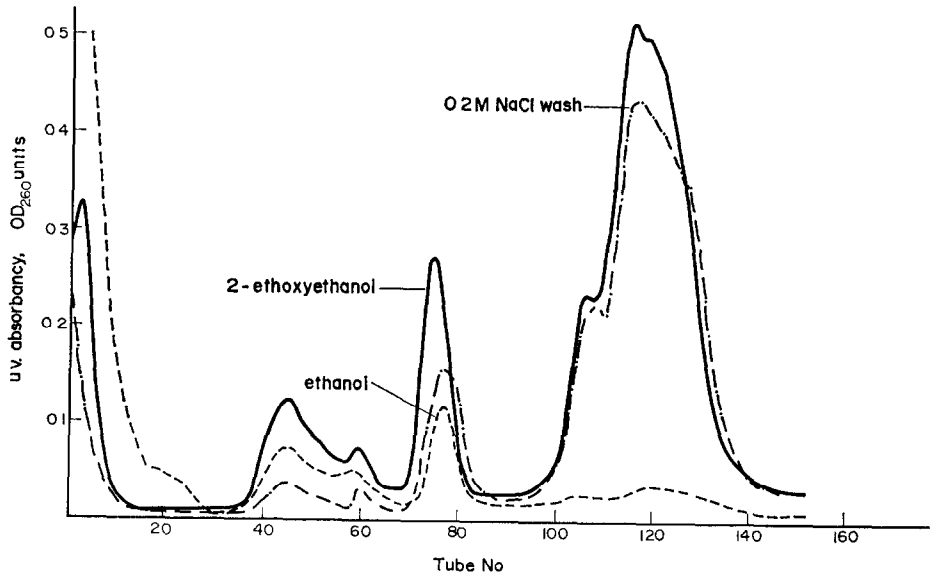


FIG. 3. MAK ELUTION PROFILES OF NUCLEIC ACIDS FROM LYOPHILIZED RED-OSIER DOGWOOD BARK TISSUE.

Nucleic acids were precipitated with 2-ethoxyethanol at 0–4° (——) or ethanol at −20° (-----). Elution profile of nucleic acids precipitated with ethanol and eluted from a MAK colum during the preliminary wash with 0·2 M NaCl (—·—).

The methods established for nucleic acid extraction from boxwood leaves and dogwood bark are presented in Scheme 1 and Scheme 2, respectively.

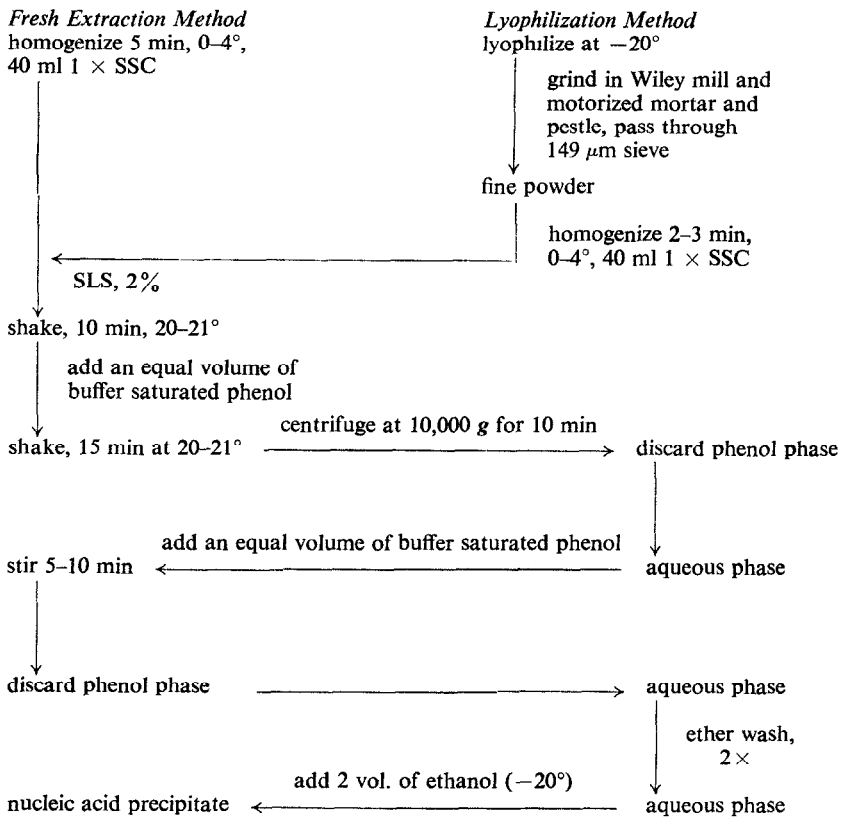
EXPERIMENTAL

Boxwood leaves were collected manually and the bark of redosier dogwood was scraped off with a sharp scalpel. Samples were either extracted immediately or stored at −20° prior to use. Lyophilization was carried out in a freezer at −20°, unless otherwise noted. Lyophilized samples were ground in a Wiley mill through a 60 mesh screen and then further ground in a motorized mortar and pestle until the material would pass through a 149 μm sieve. Homogenization of samples was done in a 150 ml flask fitted to a VirTis "45" homogenizer operated at the highest speed (45,000 rev/min). Unless otherwise noted, all homogenizations were done at 0–4° in a buffer containing 0·01 M Tris, 0·15 M NaCl, 0·015 M Na citrate, 0·01 M 2-mercaptoethanol (pH 7·7) (1 × SSC buffer) and the indicated amount of 25% sodium lauryl sulfate (SLS). In all cases, the homogenates were centrifuged at 10,000 *g* for 10 min to separate the phenol or $CHCl_3$–isoamyl alcohol (24:1, v/v) and residue from the aqueous phase. Then, the aqueous solutions containing the nucleic acids were removed with a syringe and subsequently extracted once with an equal volume of phenol for 10 min. After recentrifugation, the aqueous phase containing the nucleic acids was removed and nucleic acids were precipitated with 2 volumes of cold ethanol (−20°) in the case of boxwood and with 2 vol. of 2-ethoxyethanol (0–4°) in the case of dogwood. The precipitated nucleic acids were collected by centrifugation at 10,000 *g* for 10 min, washed twice with ethanol to remove phenol or 2-ethoxyethanol, and dissolved in a 0·05 M sodium phosphate buffer at pH 6·7.

The nucleic acid extracts were placed on columns of methylated albumin coated onto kieselguhr (MAK) according to the method of Mandell and Hershey.[22] The loaded columns were washed with 100 ml of 0·2 M NaCl in sodium phosphate buffer to remove low molecular weight contaminants. A linear gradient consisting of 425 ml of 1·25 M NaCl passing into 425 ml of 0·3 M NaCl in 0 05 M sodium phosphate buffer at pH 6·7

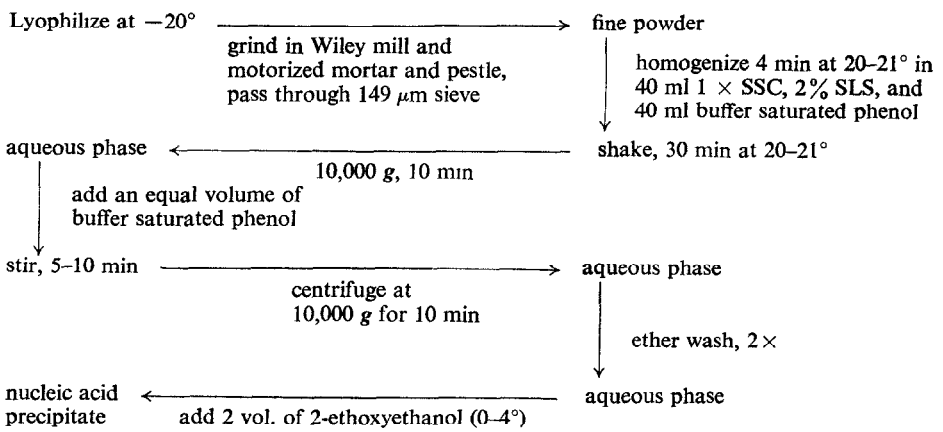
[23] J. D. MANDELL and A. D. HERSHEY, *Anal. Biochem.* **1**, 66 (1960).

BOXWOOD LEAVES



SCHEME 1. EXTRACTION OF NUCLEIC ACIDS FROM KOREAN BOXWOOD LEAVES.

DOGWOOD BARK



SCHEME 2. EXTRACTION OF NUCLEIC ACIDS FROM RED-OSIER DOGWOOD BARK TISSUE.

was then used for elution. Five-ml fractions were collected and the absorbance at 260 nm recorded. All buffers were filtered through a 0 45 μm filter prior to use. Nucleic acids were identified on the basis of their order of elution from MAK columns.

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