Nucleotides of Sugarcane: TCA-Soluble and Protein-Bound Nucleotides of Sugarcane Meristem

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INTRODUCTION

GENERAL BACKGROUND

Nucleoproteins have received much attention from biologists because of their close relationships with chromosomal materials of cell nuclei. It is widely held that chromosome nucleoproteins are directly involved in transmission of hereditary characters. Extranuclear materials also contain nucleoproteins and are believed to participate in the synthesis of cytoplasmic proteins.

Essential constituents of nucleoproteins include the acidic, non-amino-acid units which are commonly termed "nucleic acids" (*13*)[2] [3]. These acids are found in abundance among yeast and bacteria, and in the mammalian thymus gland. They are found in varying degree among numerous plant and animal sources. The viruses are appropriate examples of biological material containing nucleic acids (*16*). Of no less importance are the products of nucleic acid cleavage, commonly termed "nucleotides."

Nucleotides contain three components linked to one another, including a nitrogenous base (derived from purine or pyrimidine), a 5-carbon sugar or pentose, and phosphoric acid. Their arrangement is as follows:

Nitrogenous base — Ribose — Phosphoric acid

Nucleotides obtained from yeast nucleic acid vary as to the nature of the purine or pyrimidine derivative and in the attachment site of phosphoric acid. The 5-carbon sugar of yeast nucleotides is *d*-ribose. Hydrolysis of thymus nucleic acid, like that of yeast, also yields purines and pyrimidines in the form of nucleotides. Yet a distinctive difference between nucleotides from the two sources is the occurrence of 2-deoxy-*d*-ribose in thymus rather than *d*-ribose. It was once thought that ribose nucleic acid (RNA) was found only in plants, while deoxypentose nucleic acid (DNA) was

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[2] Italic numbers in parentheses refer to Literature Cited, pp. 226–7.

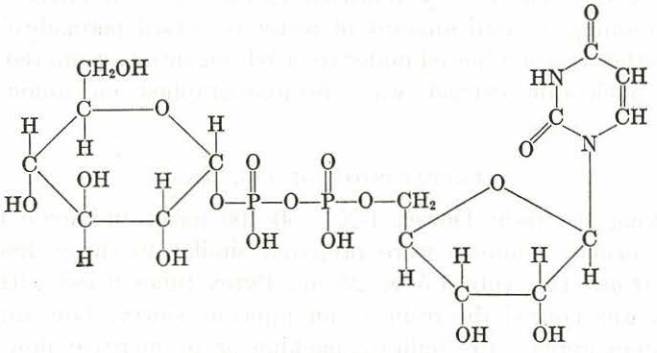
[3] The reader is referred to the extensive work by Davidson (*9*) on nucleic acids, and to the volumes edited by Chargaff and Davidson (*7*).

characteristic of animal cells. Today both types are known in virtually all kinds of cells examined.

NUCLEOTIDES IN RELATION TO SUGARCANE

Investigators dealing with higher plants must recognize both the existence and biochemical importance of nucleotides. The acids adenylic, guanylic, cytidylic, and uridylic, for example, are general constituents of viruses from tobacco, cucumber, tomato, and turnip (*17,10*). In 1929 one of the most important compounds in living matter was discovered almost simultaneously in Germany and the United States. The compound was identified as an adenine nucleotide containing three phosphoric acid groups (*5*), and is presently known as adenosine-5-triphosphate, or ATP. Other nucleotides of immeasurable importance include the electron carriers diphosphopyridine nucleotide (DPN), triphosphopyridine nucleotide (TPN), flavin adenine dinucleotide (FAD), the nucleotide derivative termed coenzyme A (CoA), and the nucleotide-containing vitamin cyanocobalamin (vitamin B_{12}).

Workers with sugarcane have a definite interest in certain derivatives of uridine-5-diphosphate (UDP). Of particular importance is UDP-glucose (UDPG), in which glucose-1-phosphate and uridine monophosphate (UMP) are joined by a pyrophosphate bond (*19*):



Uridine diphosphate glucose

Leloir and coworkers (*6,15,20*) have developed the following reactions relating UDPG to sucrose formation:

1. UTD + G-1-P $\rightleftharpoons$ UDPG + PP
2. UDPG + fructose $\rightleftharpoons$ Sucrose + UDP
3. UDPG + fructose-6-P $\rightleftharpoons$ Sucrose phosphate + UDP

The role of UDPG has also been noted by Turner (*23,24*), Alexander (*1*),

Duggar and Humphreys (*11*), and by Hatch and coworkers (*13*). UDPG is likewise credited with an active role in starch synthesis (*3*).

Nucleotides for studies such as those listed above are usually obtained commercially, and in the instance of sugarcane very little attention has been given to endogenous nucleotide constituents. The author became interested in these compounds following observations by Dr. A. Maretsky[4] that strong ultraviolet (U.V.)-absorbing materials were present in sugarcane meristem extracts. Initial efforts to isolate and identify the nucleotides of sugarcane are summarized in this paper.

MATERIALS AND METHODS

All studies were conducted with meristem tissues of the sugarcane varieties P.R. 980 and M. 336. Plants were grown in sand culture under controlled nutrient supply[5]. When these were 14 weeks of age, meristem samples were frozen in a mixture of Dry Ice and acetone, lyophilized, ground in the cold to pass a 60-mesh screen, and stored at −10° C. until needed for analysis.

PREPARATION OF ACID-SOLUBLE FRACTIONS

Four grams of meristem powder were extracted with 10-percent trichloroacetic acid (TCA) for 1 hour at room temperature (20–22° C.). Tissue debris was removed by centrifuge and washed with one volume of 10-percent TCA. TCA was removed by repeated extraction with ethyl ether, the latter containing a small amount of water to retard peroxide formation. Residual ether was eliminated under reduced pressure at room temperature, and the nucleotide extract was chromatographed on anion-exchange columns.

PREPARATION OF COLUMNS

Employing the resin Dowex 1-X4, 50–100 mesh and ionic form Cl^-, Dowex 1-formate columns were prepared similar to those described by Hurlbert *et al.* (14). Into 1.5 × 25-cm. Pyrex tubes fitted with a glass-wool disk was poured the resin as an aqueous slurry. Low air pressure was at times applied to facilitate packing or to increase flow rates. A second glass-wool disk was placed near the top and the column was washed

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[5] Nutrient concentrations expressed as milliequivalents per liter, were provided as follows: Nitrate, 10; phosphate, 6; potassium, 5; calcium, 3; magnesium, 2; and sulfate, 2. Microelements, expressed as parts per million, were supplied as follows: Boron, 0.05; copper, 0.02; manganese, 0.50; zinc, 0.05; molybdenum, 0.01; and iron, 0.50.

with 5 bed-volumes of 6*N* formic acid in 1M[6] sodium formate. This was followed by 3 bed-volumes of concentrated formic acid and finally by distilled water until the effluent achieved a pH of 6 to 7. Washing with concentrated formic acid removes colored material from the resin and reduces the U.V. light-absorption background. The considerable swelling and shrinking experienced by the resin is not detrimental (*14*), and the column may be reused after washing.

A 6-ml. sample was rinsed on to the column with water at the rate of approximately 2 ml. per minute. The column was prevented from drying by continuous application of eluting fluid, and initial effluent fractions were collected immediately after the last of the sample was absorbed on the column.

The elution technique of Hurlbert (*14*) and others (*21*) employs a gradient system which supplies continuous elutant with gradually increasing acidity. In effect, advantage is taken of the special importance of the medium's pH. In order to cause an ion-exchange separation among members of an ionic family it is essential to provide conditions under which each ion experiences a different degree of affinity for the exchanger (*8*). Assuming that among closely related nucleotides the net charge per ion is most important, and since nucleotides possess both acidic and basic groups, the pH of the medium establishes the net charge by determining the extent of group dissociation. Gradient systems can employ this principal to advantage by slowly lowering or raising the pH of the eluting fluid, and thereby selectively removing absorbed materials from the column.

During the present studies we were not equipped either with gradient facilities or with an automatic fraction collector. The following eluting systems were employed with manual collection: 1M sodium formate in 1*N* formic acid, pH 3.3; 1M sodium formate in 2*N* formic acid, pH 2.0; and 6*N* formic acid, pH 0.6. Hydrochloric acid, 0.01*N* and 0.1*N*, was used in other experiments with Dowex 1-X4 columns previously washed with 1*N* HCl, as described by Cohn (*8*).

ASSAY OF COLUMN FRACTIONS

One-milliliter samples were collected in test tubes and appropriate dilutions of 1:5, 1:30, or 1:60 were transferred to 1-cm. silica cells for U.V. analysis. A Beckman Model DB spectrophotometer equipped with a hydrogen lamp power supply was used. Optical density was determined for each sample at 260 and 275 mμ.

Peak U.V.-absorption fractions were combined and dried at room temperature under reduced pressure, or frozen in thin layers and lyophilized.

[6] The letter M designates "molar" throughout.

Dried samples were taken up in a minimum of water and clarified by centrifuge. Reducing-sugar analyses were conducted by the dinitrosalicylic acid method of Sumner (*22*), and phosphorus was measured by the phosphomolybdic acid technique (*18*). Aliquots were hydrolyzed in 1*N* H_2SO_4 (100° C. for 100 minutes) and thereafter assayed for total phosphorus. Fluorescence spectra were determined with a fluorescence attachment for the Beckman Model DU spectrophotometer. Samples of the peak absorption fractions were dried on Whatman No. 1 filter paper, cut into approximately ½ x 1-inch strips, and analyzed with the aid of a paper strip holder in the fluorescence attachment. Individual fluorescing spots were also cut from paper chromatograms and assayed in like manner.

Concentrated U.V.-absorption fractions were subjected to infrared analysis, using a Beckman Model IR-10 Infrared Spectrophotometer. Samples were either dried as films on thin sheets of plastic, or prepared as KBr pellets. Infrared studies were not entirely satisfactory owing to interference of formate in virtually all samples.

Paper chromatographic studies were conducted with concentrated U.V.-absorbing samples. The descending technique was employed in one dimension with Whatman No. 1 chromatography paper and the solvents ethanol-0.3M ammonium acetate, 2.5:1, or butanol-pyridine-water, 6:4:3. Ten to twenty λ (μliter) of cane concentrates were usually spotted, plus equivalent volumes of 0.10-percent solutions of reference nucleotides. Dried chromatograms were scanned with a Chromato-Vue apparatus equipped with both long-wave and short-wave U.V. lamps.

PROTEIN-BOUND NUCLEOTIDES

Although the TCA extraction tends to bypass proteins, we were nevertheless interested in nucleotides which might be protein-bound. Additional meristem samples were extracted with water, clarified by centrifuge, and protein was precipitated between 0 and 90-percent saturation with ammonium sulfate at pH 7.0 and 22° C. The crude protein was taken up in water and dialyzed overnight against 1 liter of distilled water. This water was retained, frozen in a thin layer and lyophilized to dryness. Residual materials were taken up in 5 ml. of water and subjected to identical analyses as described for TCA preparations.

Total nucleotide content was estimated in terms of $O.D._{260}$ units. A reference solution was prepared containing the monophosphates of adenosine, uridine, and cytidine, each at the concentration of 0.01 μmole/milliliter. One $O.D._{260}$ unit gave an optical density reading of 0.22 at 260 mμ.

Reference nucleotides and biochemicals used throughout these studies were obtained from Nutritional Biochemicals Corp., Cleveland 28, Ohio, or from P-L Biochemicals, Inc., 1037 W. Mckinley Ave., Milwaukee, Wis.

Use was made of appropriate equipment available to us and mention of a specific brand name does not imply preference or superiority of a given item over any other.

RESULTS AND DISCUSSION

ULTRAVIOLET-ABSORBING FRACTIONS DERIVED WITH FORMATE AND HCL SYSTEMS

Two U.V.-absorbing areas were quickly obtained from the resin column when formate systems of pH 3.3 and 2.0 were used. The pH 2.0 elutant gave

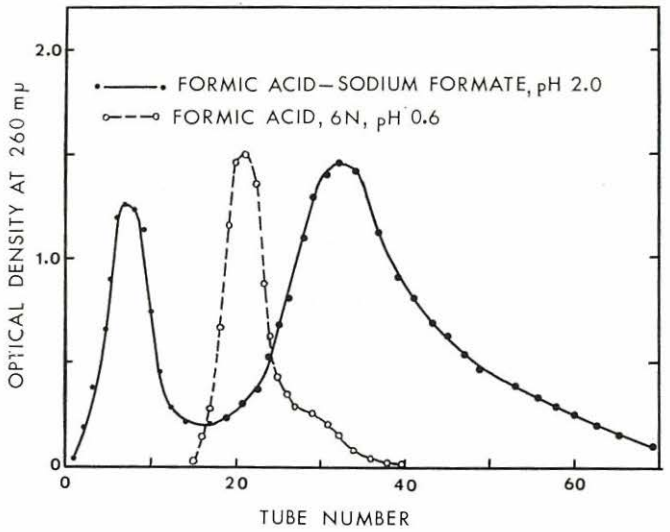

FIG. 1.—Anion-exchange chromatography of nucleotides obtained from TCA extracts of sugarcane meristem. Variety M. 336 was used. One-ml. samples were collected per tube, and aliquots were diluted 1:30 prior to U.V. analysis. 6*N* formic acid was introduced on the column at tube No. 140.

a slightly greater yield, yet continued collection up to 500 ml. did not give additional nucleotide peaks. This does not mean that further elution might not have been productive. Cohn (*8*), for example, passed up to 4 liters through a Dowex-1 column for complete separation of monoribonucleotides. This was not practical, however, with manual collection, and we followed the second nucleotide peak with a 6*N* formic acid elution. The latter gave a single additional peak. Each of the three peaks are illustrated by figure 1.

It was observed almost at once that significant differences appeared when meristem extract from another variety was chromatographed. Figure 2 demonstrates identical chromatography procedure with P.R. 980, as contrasted with the M.336 results illustrated by figure 1. Considering the

importance of nucleotides in sugar transformations, one might ponder the significance of variable nucleotide content in determining varietal sugar characteristics. P.R. 980 was used throughout most of the study. Six to eight mililiters were collected from each peak, concentrated, and referred to thereafter as F_1, F_2, and F_3.

Chromatography with HCl was less satisfactory than with formate. A single nucleotide peak was obtained in tubes 5 to 14 with 0.01*N* HCl, and

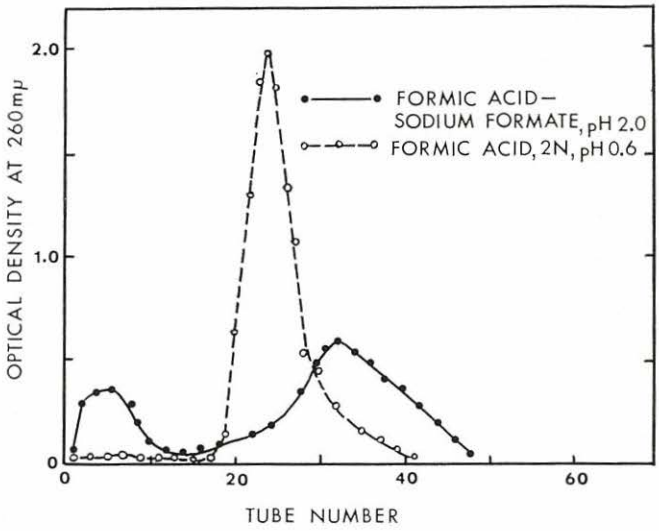

FIG. 2.—Anion-exchange chromatography of nucleotides obtained from TCA extracts of sugarcane meristem. Variety P.R. 980 was used. One-ml. samples were collected per tube, and aliquots were diluted 1:30 prior to U.V. analysis. 6*N* formic acid was introduced on the column at tube No. 140.

increasing HCl to 1.0 *N* did not yield additional U.V.-absorbing material (fig. 3). Possibly much of the material comprising F_2 and F_3 were free bases (adenine, guanine, uracil, cytosine) or ribosides and consequently had little affinity for the acid, HCl-washed exchanger.

NUCLEOTIDES FROM MERISTEM PROTEIN

Concentrated dialysate eluted at pH 2.0 from Dowex-1 formate yielded two U.V.-absorbing peaks, as did the TCA preparations, and a third peak when the column was eluted with 6*N* formic acid (fig. 4). Initial suspicion that these were identical with F_1, F_2, and F_3 was soon discontinued and the new peaks were designated F_4, F_5 and F_6.

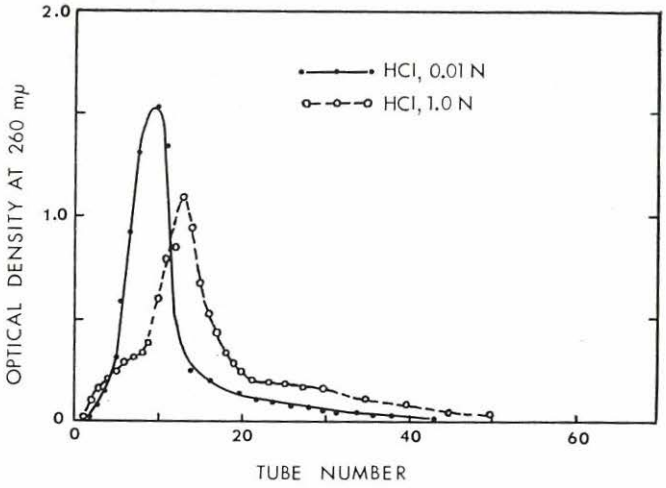

FIG. 3.—Anion-exchange chromatography, with HCl, of TCA-soluble nucleotides from sugarcane meristem. One-ml. samples were collected and diluted 1:5 prior to U.V. analysis. Variety P.R. 980 was used.

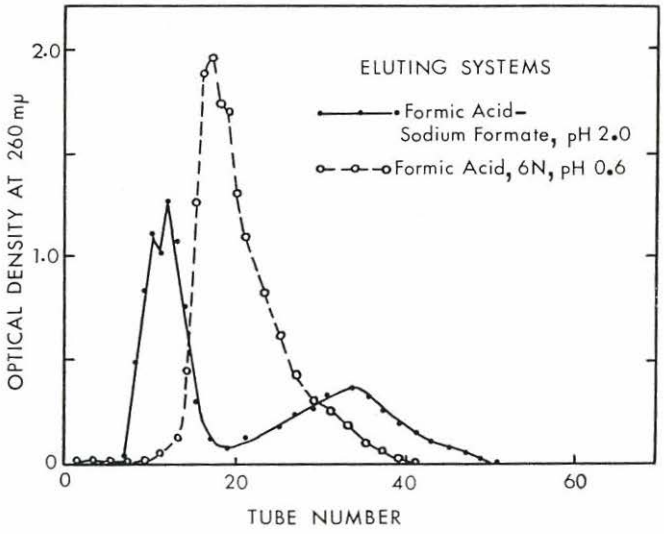

FIG. 4.—Anion-exchange chromatography of nucleotides removed from sugarcane protein by dialysis against distilled water. Dialysate was concentrated by freeze drying, rinsed on to a Dowex-1 formate column with water, and eluted with the systems indicated. One-ml. samples were collected and aliquots were diluted 1:20 prior to U.V. analysis.

CHEMICAL AND SPECTROPHOTOMETRIC RESULTS

One reason for considering the dialysate nucleotides to be distinct from those of the TCA preparation was their differing sugar and phosphorus contents (table 1). F_1 easily contained the greatest amount of sugar, whereas F_3 and F_4 were also high. Curiously, the number 60 appears as a common denominator. This may represent a single pentose molecule, with 240, 300, and 360 suggesting four-, five-, and sixfold repetitions of this sugar. Phosphorus content was highest in F_5, but each of the protein-nucleotide peaks

TABLE 1.—*Analytical data for ultraviolet-absorbing solutions derived from sugarcane meristem preparations by anion-exchange chromatography*

Data classification	U.V. absorption peak—					
	F_1	F_2	F_3	F_4	F_5	F_6
Reducing sugar[1]	370	65	298	235	63	61
P^1	1.5	10	4	26	51	17
Total P^1	2.5	11.8	6.4	26	51	17
$\frac{E_{275}}{E_{260}}$	.91	.67	1.55	.80	.64	1.53
Maximum fluorescence (μ)	450	470	450	445	470	460
Total nucleotides (O.D. 260 units/ml.)[2]	55	55	6,000	256	146	910
I.R. absorption bands (μ)	(formate)	(formate)	(formate)	1,070–1,170[3]	610, 630,[3] 1,110, 1,190	1,170,[4] 1,270

[1] Milligrams $\times 10^3$ per milliliter of combined peak fractions.

[2] Nucleotide content was computed for the milliliter giving maximum U.V. absorption within each peak.

[3] KBr pellet.

[4] Film on plastic.

contained greater phosphorus than the TCA preparation. Very little additional phosphorus was released by the acid hydrolysis treatment.

Extinction ratios 275 mμ:260 mμ are virtually identical for peaks F_2 and F_5 at close to 0.65 (table 1). These suggest the presence of uridine (E_{275}: E_{260} = approximately 0.60). Extinction ratios for F_3 and F_6 are likewise almost identical at about 1.54, which, in turn, implicates cytidine (E_{275}: E_{260} = 1.2–2.0).

Fluorescence spectra were generally weak with the exception of F_3 (fig. 5). Maximum fluorescence for all samples lay between 445 mμ and 470 mμ (table 1). Fluorescence peaks were identical for F_2 and F_5, as were extinction ratios. However, both the phosphorus and total nucleotide data indi-

cate that F_2 and F_5 represent different types and quantities of U.V.-absorbing material.

Total nucleotide content was by far the highest in F_3. It is quite possible that had the pH 2.0 eluting system been passed through the ion-exchanger over a prolonged period additional peaks would have been recovered. The followup with 6*N* formic acid may have simply gathered together a number of smaller, well-retained nucleotide groups and massed them into a single peak (F_3).

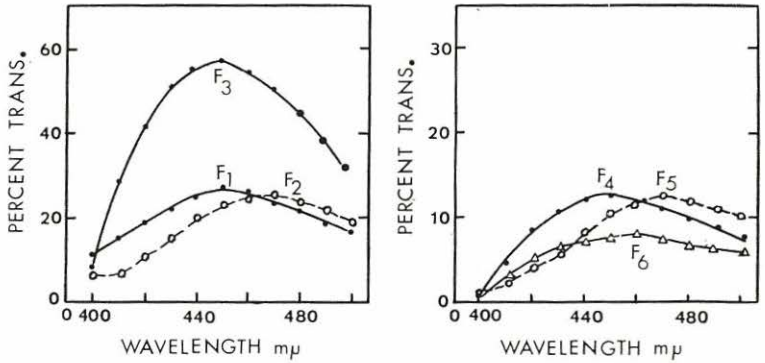

FIG. 5.—Fluorescence spectra of sugarcane nucleotide preparations obtained by anion-exchange chromatography. Fractions F_1, F_2, and F_3 represent TCA-soluble material from lyophilized meristem. Fractions F_4, F_5, and F_6 were removed from meristem protein by 12-hour dialysis against distilled water.

PAPER CHROMATOGRAPHY

Nucleotides

Chromatography experiments were not entirely conclusive, yet a number of constituents were isolated from peak column fractions, and tentative identification was achieved for several nucleotides and sugars. The greatest problem arose from heterogeneity of peak constituents which yielded masses of stain material, even after 2-dimensional separation and prolonged irrigation periods. Figure 6 illustrates a typical 8-hour separation of F_1–F_6 with ethanol-ammonium acetate, 2.5:1. Exaggerated extension of spot fronts was common with this solvent. The problem was alleviated by use of other solvents, especially butanol-pyridine-water, 6:4:3 *v/v*, and by computing Rf values on the basis of the spot center of gravity rather than front, *i.e.*,

$$\text{Rf} = \frac{\text{Distance traveled by spot center}}{\text{Distance traveled by solvent front}}$$

Figure 7, A, shows constituents of F_1, F_2, and F_3 chromatographed simul-

taneously with 10 μg. of the compounds uridine, adenine, guanosine, cytidine, cytosine, thymidine, thymine, and uracil. Scanned with a U.V.-light source, at least four constituents were visible from F_3, two of which (spot Nos. 7 and 8) exhibited strong visible fluorescence. Spot No. 9 is very nearly identical to uridine, and possible uracil (Rf No. 9 = 0.74; uridine = 0.75; uracil = 0.77). Spot No. 8 apparently represents an unresolved mass, the most mobile portion of which has a spot front similar to adenosine.

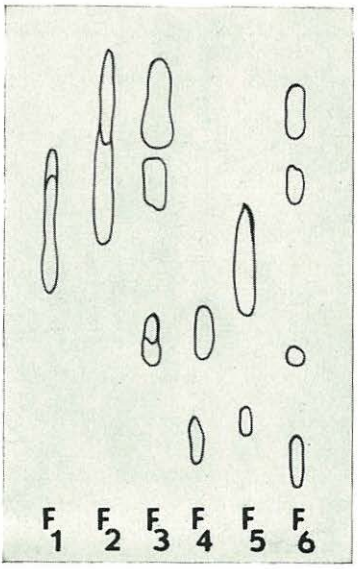

FIG. 6.—Paper chromatography of sugarcane nucleotide preparations. F_1, F_2, and F_3 represent TCA-soluble fractions from lyophilized meristem. F_4, F_5, and F_6 represent fractions derived from meristem protein by dialysis against water. Irrigation time was 8 hours, with the solvent ethanol-0.3M ammonium acetate, 2.5:1.

Both uridine and adenosine are factors of immeasurable importance to sugarcane and are among the first to be sought in a nucleotide study. With the exception of spot No. 5, the unknown materials from F_1 and F_2 appear unrelated to the tested reference compounds.

Figure 7, B illustrates F_4, F_5, and F_6 chromatographed with the same standards discussed above. The four compounds from F_6 are similar to those of F_3, but none fluoresce brightly. Spot No. 6, which might have been uridine, more nearly resembles uracil. Spot No. 5 is undoubtedly adenosine (Rf for both = 0.66). Spot No. 4, Rf 0.59, is apparently cytidine (Rf cytidine = 0.58). Cytidine was anticipated in both F_3, and F_6 on the basis of extinction ratios (table 1).

The acids adenylic, uridylic, and cytidylic, which are relatively immobile in the butanol-pyridine-water solvent, did not appear among any of the sugarcane preparations. Thus the adenosine, uridine, and cytidine discussed

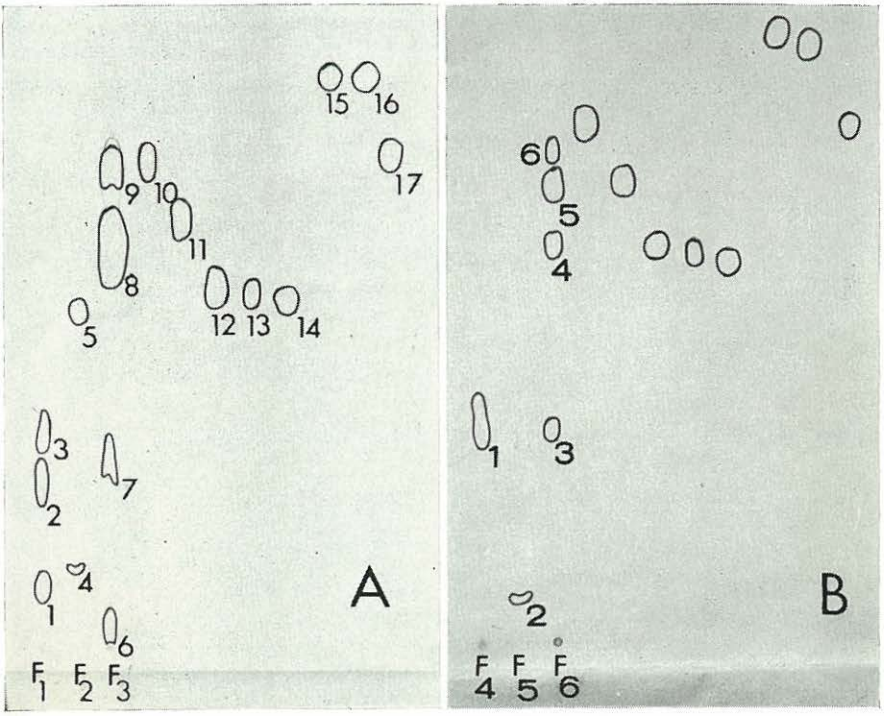

FIG. 7.—Paper chromatography of sugarcane nucleotide preparations with 10 μg.-quantities of known U.V.-absorbing compounds. Reference compounds are numbered as follows in chromatogram A: 10, uridine; 11, adenosine; 12, cytidine; 13, guanosine; 14, cytosine; 15, thymidine; 16, thymine; and 17, uracil. The same reference materials were used for chromatogram B. F_1, F_2, and F_3 (chromatogram A) represent TCA-soluble materials, and F_4, F_5, and F_6 (chromatogram B) represent materials dialysed from meristem protein. Irrigation time was 20 hours, with the solvent butanol-pyridine-water, 6:4:3.

rather loosely above as "nucleotides" might more correctly be termed nucleosides. Apparently a partial hydrolysis occurred during preparation of the nucleotide samples, probably yielding phosphoric acid and nucleosides in which the base was still united with the sugar. Severe hydrolysis should yield the purine or pyrimidine, phosphoric acid, and furfural from ribose degradation (*12*).

Sugars

Colorimetric analyses indicated high sugar concentrations in F_1, F_3, and F_4 (table 1). Sugars were investigated by paper chromatography using the silver nitrate dip method described previously (4). The solvent butanol-pyridine-water was used for all sugar chromatography.

Figure 8 illustrates sugar groups found in the cane nucleotide preparations. Their chromatographic relationships with common reference sugars

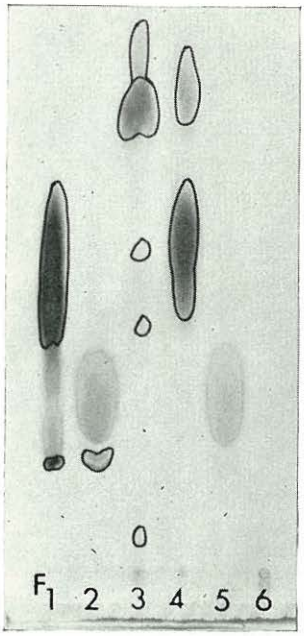

FIG. 8.—Paper chromatography of sugar constituents of cane nucleotide preparations. Irrigation time was 12 hours, with the solvent butanol-pyridine-water, 6:4:3. Stains were developed by the silver nitrate dip technique.

are presented in figure 9. Two intense but probably identical sugar masses are clearly evident in figure 8. All spots representing sugars are circled with "magic marker", including a very immobile and faintly staining constituent of F_3 believed to be UDPG. F_2 and F_3 yielded identical stained areas which probably represent hexose phosphates. No sugars were detected at all from F_6, even though chemical analysis indicated the presence of reducing sugar (table 1).

The chromatogram shown in figure 9,A includes the TCA-soluble preparations (F_1, F_2, F_3) plus 40 μg. quantities of glucoseamine, UDPG, glucose, maltose, ribose, and galactose. Obviously, the sugar spot No. 2 from F_1

could include glucose, glucoseamine, maltose, and galactose. Glucose and glucoseamine are most probable since neither maltose nor galactose are of significance in cane. Spot No. 5 from F_3 is apparently UDPG (Rf No. 5 = 0.074; Rf UDPG = 0.076). Rf for spot No. 3 (F_3) is 0.065. Another UDP

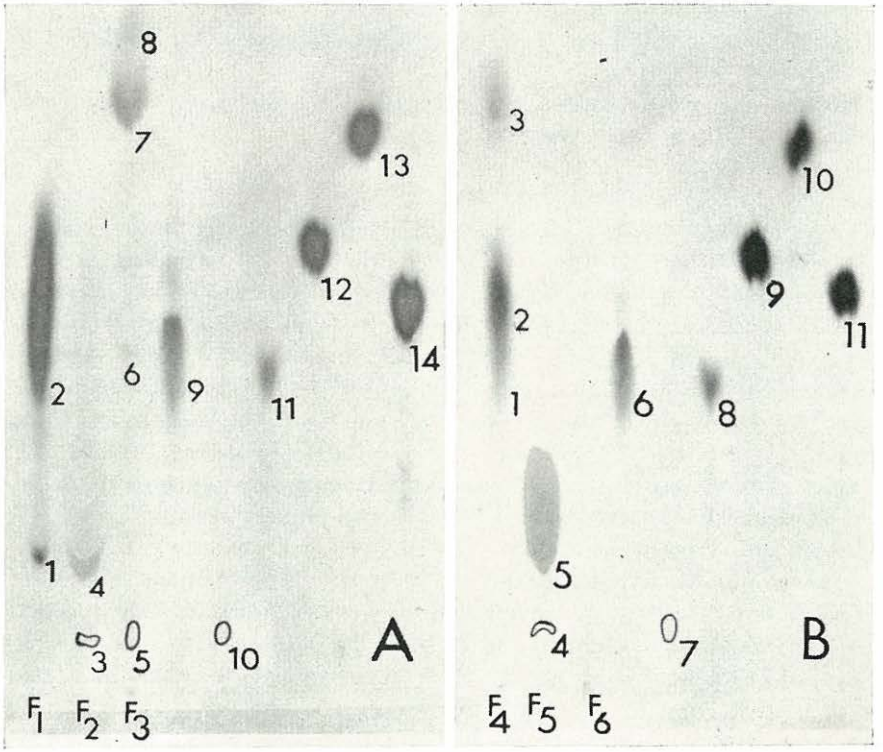

FIG. 9.—Sugar constituents of cane nucleotide preparations chromatographed with 40-μg. quantities of known sugars. Reference compounds in chromatogram A are numbered as follows: 9, glucoseamine; 10, UDPG; 11, glucose; 12, maltose; 13, ribose; and 14, galactose. Identical reference compounds are used in chromatogram B, with No. 6 representing glucoseamine, No. 7 UDPG, etc. Irrigation time was 20 hours, with the solvent butanol-pyridine-water, 6:4:3.

derivative such as UDP-glucuronic acid or UDP-acetylgalactosamine might also be present. The principal mobile sugar of F_3 (spot No. 7) is probably a pentose. It does not accurately match ribose (Rf No. 7 = 0.76; Rf ribose = 0.73), yet both 7 and 8 logically represent ribose derivatives or products. Spot No. 7 superimposes precisely area No. 9 (uridine), figure 7,A.

Chromatography of protein-derived nucleotides permitted a faint distinction between two staining areas in the sugar mass of F_4 (fig. 8). These spots are not clear enough for accurate Rf determination, but No. 1 most nearly matches glucoseamine, and No. 2 is highly suggestive of galactose. Spot No. 3 (F_4) is probably a ribose derivative. No. 4 (F_5, Fig. 9,B) has an Rf value identical with UDPG, although the spot is malformed at the front of a nonstaining patch of formate. This is either UDPG or a related UDP derivative.

No evidence of coenzymes I and II, or of the flavin nucleotides, was encountered during these experiments.

TCA-SOLUBLE *VS.* PROTEIN-BOUND NUCLEOTIDES

To some degree the U.V.-absorbing material removed from protein seems identical with that obtained by TCA extraction. In particular, the 6*N* formic acid wash, represented by F_3 and F_6, yielded nearly identical chemical data and fluorescing spots on paper chromatograms. Staining properties of F_2 and F_5, and of F_1 plus F_4, are somewhat similar on paper chromatograms. On the other hand, protein-bound materials definitely include a riboselike sugar which readily emerges from the anion-exchange column (fig. 9,B, spot No. 3). Conversely, the TCA extracts contain a less mobile, heavily staining sugar (fig. 9,A, spot No. 1) which was not present in the protein-derived material. So long as such compounds are unknown their full significance remains obscure.

From a quantitative standpoint the TCA extraction method should not be considered adequate for sugarcane nucleotides. Future studies should also include protein-bound materials from the standpoint of possible enzyme cofactors, activators, or inhibitors. In particular, the cane invertases found concentrated in meristem and immature storage tissue are severely inactivated by dialysis (*2*). Our failure to detect such compounds as DPN, TPN, FAD, and CoA does not mean at this early stage that they are definitely not there. Investigators interested in cane enzymes as well as nucleotide constituents may find meristem protein a valuable source, particularly in view of the very high enzyme activity and abundant protein found in this tissue.

IMPORTANCE OF CANE NUCLEOTIDE RESEARCH

It was already pointed out that nucleotide data may be of value in determining the causes of varietal characteristics. Materials such as the UDP derivatives are of special interest because of their roles in sucrose synthesis. Yet nucleotide research might well be extended to other problems confronting cane workers. For example, to what degree are nucleotides involved in virus disorders, or related to host susceptibility or expression of symp-

toms? How do nucleotides affect sugar production under extremes of temperature, nutrient supply, water supply, and light intensity? To what extent is the *type* of nucleotide important as opposed to nucleotide *content* or *synthesis*? Undoubtedly the incorporation of nucleotide data might greatly increase the scope, interpretation, and ultimate value of sugarcane research.

SUMMARY

Initial studies of sugarcane nucleotides were conducted by anion-exchange and paper chromatographic techniques. Ultraviolet-absorbing materials were extracted from lyophilized meristem tissues with trichloroacetic acid and absorbed on columns of Dowex-1 formate. Nucleotides were removed in two peaks with a 2*N*, formic acid-sodium formate eluting system at pH 2.0. A third nucleotide peak was obtained with 6*N* formic acid, pH 0.6.

Meristem protein was extracted with water, precipitated between 0 and 90-percent saturation by ammonium sulfate, and dialyzed against distilled water. Dialysate was concentrated by lyophilization and chromatographed on Dowex-1 formate. Again, two U.V.-absorbing peaks were gained with the pH 2.0 eluting system, and a third peak with 6*N* formic acid. U.V.-absorbing materials were obtained with HCl (0.01 *N* and 1.0 *N*) from both TCA and protein preparations, but HCl was less satisfactory than the formate-formic acid systems.

Analyses of U.V.-absorbing concentrates included reducing sugar, phosphorus, U.V.-extinction ratios, fluorescence spectra, total nucleotides, and infrared absorption. Paper chromatograms were prepared for nucleotide and sugar constituents of peak fractions. Nucleosides identified chromatographically were uridine, adenosine, and cytidine. Uracil was possibly present, while guanosine and cytosine were in doubt. Glucoseamine was apparently present in the TCA extracts. Uridine diphosphate glucose was tentatively identified in both preparations, as was ribose or a ribose derivative.

No evidence for coenzymes I and II, coenzyme A, or flavin nucleotides was obtained. Significance of nucleotide research in sugarcane is briefly discussed.

RESUMEN

Se llevaron a cabo estudios preliminares con los nucleótidos de la caña de azúcar usando las técnicas de intercambio aniónico y cromatografía en papel. Se extrajo con ácido tricloroacético material absorbente de luz ultravioleta del tejido meristemático liofilizado, y se absorbio en columnas de resina de formato de Dowex-1. Los nucleótidos se separaron en dos puntos de absorción máxima usando un sistema de elución combinado de ácido fórmico y formato de sodio, ambos 2*N*, y a un pH de 2.0. Se obtuvo un

tercer punto de absorción máxima de nucleótidos al eluir con ácido formico 6*N*, y a un pH de 0.6.

Se extrajo la proteína del meristemo con agua, se precipitó a una saturación entre 0 y 90 por ciento con sulfato amónico, y se dializó con agua destilada. El dializado se concentró liofilizándolo y se cromatogramó a través de la columna de formato de Dowex-1. Nuevamente se obtuvieron dos puntos de absorción máxima de luz ultravioleta con el sistema de elución de pH de 2.0, y un tercer punto de absorción máxima con el ácido formico 6*N*. Se extrajeron nucleótidos con HCl (0.01*N* y 1.0*N*) de preparaciones acuosas de proteínas y de preparaciones con TCA, pero con resultados menos satisfactorios que los obtenidos mediante los sistemas de formatos y ácido fórmico.

Los análisis de los concentrados absorbentes de luz ultravioleta incluyeron azúcares reductores, fósforo, la proporción de extinción de luz ultravioleta, el espectro de fluorescencia, los nucleótidos totales y la absorción de luz infrarroja. Se prepararon cromatogramas en papel para las fracciones máximas de los componentes de los nucleótidos y los azúcares. Se identificaron cromatográficamente los nucleósidos uridina, adenosina y citidina. La presencia del uracilo fué casi positiva mientras que la de la guanosina y la citosina estuvo dudosa. Aparentemente se encontró la glucosamina en los extractos hechos con TCA. En ambas preparaciones se identifico tentativamente uridina difosfato de glucosa y también la ribosa ó un derivado de la ribosa.

No se obtuvo evidencia de la presencia de las coenzimas I y II, la coenzima A ó los nucleótidos de flavina. Se discute brevemente la importancia de la investigación de los nucleótidos en la caña de azúcar.

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