melted at 196–197° after recrystallizations from 90% acetic acid and finally ethyl acetate. This derivative failed to depress the melting point of an authentic sample of a 2,4-dinitrophenylhydrazone derivative of 2-benzoylpyridine (m.p. 196–197°). However, this represents only a trace identification of 2-benzoylpyridine since the fraction from which it was obtained contained primarily 4,4'-dipyridyl.

Discussion of infrared results. The fractions obtained from the Het BR of *sec*-butyl acetate, *n*-amyl acetate, 2-ethylhexyl acetate, and ethyl propionate with pyridine using aluminum gave picrate and 2,4-dinitrophenylhydrazone derivatives. However the purification of these derivatives failed to yield constant melting solids, and since the yields were low, attempts to separate the isomers proved to be impractical. Therefore infrared spectra of the mixtures presumed to be 2- and 4-acetylpyridine and 2- and 4-propionylpyridine were obtained.

The characteristic absorption peak for the carbonyl band was split in these spectra, more than likely due to the presence of the 2- and 4-isomers. The absorption frequencies observed for a mixture of 2- and 4-acetylpyridine were 1655 cm.$^{-1}$ (6.04 μ) and 1680 cm.$^{-1}$ (5.94 μ), and for a mixture of 2- and 4-propionylpyridine 1660 cm.$^{-1}$ (6.02 μ) and 1685 cm.$^{-1}$ (5.93 μ). These absorption values check with those listed in the literature for carbonyl bands present in aryl alkyl ketones (1700–1680 cm.$^{-1}$).[23]

The characteristic peaks for the pyridine nucleus were also present in these spectra. Thus the mixture of 2- and 4-acetylpyridines showed absorption at 785 cm.$^{-1}$ (12.72 μ) and 746 cm.$^{-1}$ (13.40 μ), while the mixture of 2- and 4-propionylpyridine showed absorption at 835 cm.$^{-1}$ (11.99 μ) and 760 cm.$^{-1}$ (13.18 μ). These strong bands have been shown to originate in the out-of-plane vibrations of the unsubstituted hydrogen atoms of the ring.[24]

Thus we conclude from these spectra that acylation of the pyridine nucleus has occurred yielding mixtures of the 2- and 4-pyridyl substituted ketones.

LAFAYETTE, IND.

(23) L. J. Bellamy, *The Infra-Red Spectra of Complex Molecules*, Methuen and Co. LTD., 1956, p. 114.

(24) See reference 22, p. 235.

[CONTRIBUTION NO. 1454 FROM THE STERLING CHEMISTRY LABORATORY AND FROM THE BINGHAM OCEANOGRAPHIC LABORATORY, YALE UNIVERSITY]

Contributions to the Study of Marine Products. XLV. Sponge Nucleic Acids*,[1]

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Nucleic acids have been isolated from sixteen different species of sponges, and all have been degraded to the nucleosides. All pentose nucleic acids gave the four usual ribonucleosides, and all deoxypentose nucleic acids gave the four usual deoxyribosides. There were no detectable quantities of arabinosides or other unusual nucleosides among the hydrolysis products. The nucleic acids of *Cryptotethia crypta* were exceptional in their extraordinarily low content of ribonucleic acid. The significance of these observations has been discussed.

It has been shown in previous papers of this series that extended acetone extraction of the Caribbean sponge, *Cryptotethia crypta*, affords a mixture of three unusual nucleosides which have been identified as 3-β-D-arabofuranosylthymine (spongothymidine),[3] 3-β-D-arabofuranosyluracil (spongouridine),[3] and 9-β-D-ribofuranosyl-2-methoxyadenine.[4] Some earlier observations[5] had pointed to the possibility that these new nucleosides are products of the autolysis of more complex units such as nucleic acids.

To investigate this attractive possibility we began several years ago a systematic study of sponge nucleic acids of which previous knowledge was altogether lacking. Suitable methods for the isolation and examination of the nucleic acids were developed on the common sponges of Long Island Sound rather than the more promising but less accessible Caribbean sponge, *Cryptotethia*. This sponge and other species were subsequently collected near the island of Bimini.[6] They were quickly frozen and kept in this state until shortly before their extraction. A third group of sponges was collected in the Bermudas[6] and extracted there while still quite fresh. Altogether sixteen different species were investigated which included representatives of the three well-known groups, the keratineous, siliceous, and calcareous sponges. All of these sponges had previously been investigated in this laboratory for other reasons, but with the exception of *Cryptotethia*, none of them had been observed to afford the unusual nucleosides.

The nucleic acids were isolated and characterized by modifications of procedures well described in the literature. Most of them were patterned after the

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(3) W. Bergmann and D. C. Burke, *J. Org. Chem.*, 20, 1501 (1955).

(4) W. Bergmann and M. F. Stempien, *J. Org. Chem.*, in press.

(5) W. Bergmann and R. J. Feeney, *J. Org. Chem.*, 16, 981 (1951).

(6) The authors express their sincere gratitude to the directors of the Lerner Marine Laboratory, Bimini, Bahamas, and of the Bermuda Biological Station for their most valuable help.

TABLE I
SPONGE NUCLEIC ACIDS

Order	Species	Origin	Method[a]	Yield[b]	Ultraviolet Absorption λ_{max}	Ultraviolet Absorption $E^{1\%}_{1\ cm.}$	DNA	RNA
Keratosa	*Ircinia fasicu-lata*	Bermuda 1956	II	40	257 mμ	134.7	+?[c]	+++
	Dysidia craw-shayi	Bermuda 1956	II	150	258 mμ	173.5	++	+++
	Dysidia etheria[d]	Bermuda 1956	I		257 mμ	144.0	+	+++
	Ianthella ardis	Bermuda 1956	II	65	259 mμ	97.0	+	+++
	Verongia fistu-laris	Bimini 1954	II	130	259 mμ	57.5	+?[c]	+++
Poecilosclerina	*Tedania ignis*	Bermuda 1956	II	120	257 mμ	63.8	−	+++
	Microciona prolifera	Long Island 1953	IVa				+	+++
Halichondrina	*Halichondria panicea*	Long Island 1953	I	105	258 mμ	230.0	−	+++[e]
Hadromerina	*Spheciospongia vesparia*	Bimini 1954	I	40	259 mμ	73.0	+	+++
	Spheciospongia othello	Bermuda 1956	II	45	259 mμ	98.0	+?[c]	+++
	Terpios fugax	Bermuda 1956	II	155	257 mμ	148.0	+?[c]	+++
Epipolasida	*Cryptotethya crypta*	Bimini 1954	I	30	259 mμ	59.6	++	+?
			III	45	259 mμ	60.5	++	+?
			IVb	60	259 mμ	60.0	++	+?
	Tethya actinia	Bermuda 1956	II	40	257 mμ	105.0	+?[c]	+++
Choristida	*Cinachyra cavernosa*	Bermuda 1956	II	40	260 mμ	63.4	+	+++
Carnosa	*Chondrilla nucula*	Bimini 1954	IVa				+	+++
Asconosa	*Leucetta flori-dana*	Bermuda 1956	II	70	258 mμ	152.0	+	+++

[a] Full details of these extraction methods are given in the experimental section. [b] Milligrams of nucleic acid–polysaccharide per 250 grams of wet sponge. [c] These preparations gave very weak positive reactions in the Dische test for deoxypentose. The amount of DNA was too small to allow its identification by degradative methods. [d] This sponge was investigated by Mr. Joel S. Kovel. [e] This was the purest nucleic acid obtained. Found: P, 7.1%; N, 12.3%; $\epsilon\ (P)_{258}$, 10,000.

method used by Dutta, Jones, and Stacey[7] in the isolation of bacterial nucleic acids, which involves precipitations with cetyltrimethylammonium bromide. In a few cases the nucleic acids were isolated by the older method of the alcohol precipitation of the sodium salts. The deproteinization was accomplished with chloroform,[8] followed on one occasion by phenol.[9]

The acids thus obtained were more or less heavily contaminated with polysaccharides. Only in the case of the abundant Long Island sponge, *Halichondria panicea*, was this impurity largely removed, and a nucleic acid was obtained with a phosphorus and nitrogen content of 7.1% and 12.3% respectively. Otherwise extensive removal of the polysaccharides was not attempted for reasons of economy. At this stage of the investigation we were less concerned with the over-all constitution of the nucleic acids than with the structure of the individual nucleosides to be derived from them. A judicious choice of methods generally avoided the interference by polysaccharide degradation products in the identification of the bases and carbohydrates derived from the nucleic acids.

Table I lists the sponges which are included in the present investigation, their classification, origin, and the nucleic acid material obtained from them. In cases where the total nucleic acids showed a negative or a faint Dische-test for deoxypentose nucleic acids, they were hydrolyzed by both unfractionated snake venom to nucleosides and by acids to the purine bases and pyrimidine nucleotides. The hydrolyzates were chromatographed on paper against authentic markers in the conventional manner. In other cases, where significant amounts of deoxypentose were indicated, the pentose nucleic acids were first hydrolyzed with alkali to the nucleotides and these in turn to nucleosides with prostatic phosphatase. The nucleosides were then chromatographed on paper as before.

The spots on the chromatograms were eluted, and the ultraviolet spectra of the eluted fractions determined. For further identification the electro-

(7) S. K. Dutta, A. S. Jones, and M. Stacey, *Biochim. et Biophys. Acta*, **10**, 613 (1953).
(8) M. G. Sevag, D. B. Lackman, and J. Smolens, *J. Biol. Chem.*, **124**, 425 (1938).
(9) K. S. Kirby, *Biochem. J.*, **64**, 405 (1956).

TABLE II

ACID DEGRADATION OF SPONGE RIBONUCLEIC ACIDS

Sponge	Adenine	Guanine	Cytidylic Acid	Uridylic Acid	Purines / Pyrimidines
Halichondria panicea	10.0	20.1	11.0	11.3	1.35
Verongia fistularis	10.0	18.5	11.0	8.7	1.45
Spheciospongia othello	10.0	16.1	8.8	8.2	1.53
Tedania ignis	10.0	20.0	10.6	7.5	1.66
Terpios fugax	10.0	16.8	10.0	6.8	1.60
Ircinia fasciculata	10.0	17.8	10.0	9.6	1.42
Tethia actinia	10.0	15.3	8.5	7.2	1.61

phoretic mobilities in a borate buffer (*p*H 9.3) of the sponge nucleosides were compared with those of authentic nucleosides.[10] Such a comparison is most important because on paper chromatograms uridine and spongouridine, for example, show the same R_f value although one is a riboside and the other an arabinoside.[11] In a borate buffer, however, the mobility of the riboside is substantially greater than that of the arabinoside. When tested by this sensitive method none of the nucleosides gave evidence of the presence of arabinosides. For final identification at least one of the nucleosides from every nucleic acid was hydrolyzed, and the liberated carbohydrate identified chromatographically. All sponge pentose nucleic acids afforded only the four familiar ribonucleosides, guanosine, adenosine, uridine, and cytidine, see Table II.

The mixtures of deoxynucleic acids and polysaccharides were hydrolyzed with deoxyribonuclease and snake venom, and the hydrolyzates chromatographed on paper against authentic deoxyribosides. As before, the spots were eluted, and the absorption spectra of the eluted fractions determined. In all cases the usual unity proportions of purines to pyrimidines were observed; see Table III. In separate hydrolyses by formic acid the free bases were prepared and identified. The only carbohydrate obtained from any of the deoxynucleoside mixtures was 2-deoxyribose. The deoxynucleic acids from sponges, like those from other sources therefore afford the 2-deoxyribosides of adenine, guanine, thymine, and cytosine.

5-Methylcytosine has been observed in the hydrolyzates of nucleic acids from several invertebrates. Wyatt[12] obtained it from the locust and sea urchin sperm, and Rosedale[13] has stated that the nucleic acids of termites and the larvae of certain flies give about equal quantities of cytidylic and 5-methylcytidylic acid but no uridylic acid. Conscious of these observations we have looked for 5-methylcytosine in the hydrolyzates of sponge nucleic acids, but have not found any.

The results of the present study therefore show that on the whole the nucleic acids do not differ significantly in their over-all composition from other pentose or deoxypentose nucleic acids. Some rather unusual features, however, were noticed among the nucleic acids from *Cryptotethia crypta*, the only sponge which has so far given the unusual nucleosides. As has been stated before, the sponge nucleic acids appear to be quite intimately associated with polysaccharides. This is particularly evident in the case of *Cryptotethia*, where three different methods of isolation gave products of an almost identical carbohydrate content of 75% as determined by optical density measurements. The two fractions were not separable in the ultracentrifuge at 59,780 r.p.m.[14]

Among the nucleic acids of all other sponges the proportion of ribonucleic acid was always found much greater than that of deoxyribonucleic acid; in fact several samples contained only minute quantities of the latter. This is the commonly encountered quantitative relationship between the two acids. In the case of the nucleic acids from *Cryptotethia crypta*, however, the relationship was found to be quite reversed. Here, the ribonucleic acid represented less than 4% of the total nucleic acid fraction. The amount was so small that it could have been derived not from the sponge itself but from other form of life to be found on it.

It should be mentioned here, however, that for technical reasons the *Cryptotethia* sponges from which the nucleic acids had been isolated had to be kept in cold storage for more than two years before the beginning of the first extraction. The low ribonucleic acid content may thus be attributable to enzymic action over this period. Yet in all other sponges which had been stored similarly for an equal length of time, the ribonucleic acid did not differ significantly from that observed in fresh sponges. It would seem to be more than a coincidence that this very low ribonucleic acid content is found solely in *Crypototethia*, the only sponge which has so far yielded free nucleosides in such extraordinary amounts and of such unusual struc-

(10) D. C. Burke, *Chemistry & Industry*, 1510 (1954).

(11) D. M. Brown, A. R. Todd, and S. Varadarajan, *J. Chem. Soc.*, 2388 (1956). These authors differentiate uridine and spongouridine on a paper chromatogram by means of the periodate-Schiff spray reaction.

(12) G. R. Wyatt, *Biochem. J.*, **48**, 581 (1951).

(13) J. L. Rosedale, *J. Entomol. Soc., S. Africa*, **11**, 34 (1948).

(14) The authors are indebted to Prof. S. J. Singer of this department for his help in this matter.

ture. There may exist therefore in this particular sponge a metabolic aberration which diverts most of the normal intermediates of ribonucleic acid synthesis to the formation of free spongothymidine, spongouridine, and spongosine or perhaps to an unstable substance of greater complexity incorporating these nucleosides. Examination of aqueous extracts of *Crypotothethia* has not so far provided any evidence for the presence of the phosphates of the three unusual nucleosides or of larger units, but this important problem will be further pursued as soon as facilities for the speedy extraction of the living sponge have become available.

EXPERIMENTAL

Isolation of nucleic acids. All operations were carried out in a cold room at 3° except in cases involving the use of 1% or stronger cetyltrimethylammonium bromide solutions. At this temperature and concentration the detergent is prone to precipitate, and these operations were therefore carried out as speedily as possible at room temperature. Table I lists the species of sponges examined in the present investigation, their classification according to de Laubenfels[15] and their origin. It also includes the extraction procedures used for each of the various species together with the respective yields, the ultraviolet absorption data of the products, and an indication of their content of deoxyribose nucleic acid.

The methods I and II (Table I) are modifications of the techniques used by Dutta, Jones, and Stacey[7] in the isolation of bacterial nucleic acids. The methods III and IVa and b are based on older and more commonly used procedures.

Method I. The frozen sponge was cut into small pieces and homogenized in a high-speed, rotary blendor for about 3 min. with twice its weight of 0.01*M* sodium arsenate solution. The homogenate was diluted with six volumes of water, allowed to stand for 24 hours at 3°, strained through a cheesecloth, and centrifuged at 1100 r.p.m. Cetyltrimethylammonium bromide[16] was gently stirred into the supernatant solution until precipitation of the cetyltrimethylammonium salts was complete. The suspension was kept at 3° for 15 hr., centrifuged, and the resulting pellet was washed twice with water. The washings were discarded, and the pellet thoroughly extracted with *M* sodium chloride solution, and the extract centrifuged. The liquid was diluted with water to 0.25*M* sodium chloride, and the resulting precipitate redissolved as far as possible in *M* sodium chloride and reprecipitated by diluting the centrifuged solution to 0.25*M*. This procedure was repeated until the precipitated cetyltrimethylammonium salts became completely resoluble in *M* sodium chloride. The clear solution which was eventually obtained was deproteinized according to Sevag's method,[8] and the nucleic acids were precipitated from the protein-free solution by addition of two volumes of 95% ethanol. The precipitated material was collected by centrifugation, washed successively with 75% ethanol, 95% ethanol, and acetone, and finally dried *in vacuo*. For further purification, the material was redissolved in a 1% solution of cetyltrimethylammonium bromide in *M* sodium chloride (1 ml. of this solution per mg. of nucleic acid) and precipitated as the cetyltrimethylammonium salt by dilution of the solution to 0.3*M* sodium chloride. The salt thus obtained was reprecipitated twice more from *M* sodium chloride solution containing 1% cetyltrimethylammonium bromide by dilution with water, and was then reconverted into the sodium nucleate by alcohol precipitation from its solution in *M* sodium chloride. An aqueous solution of the sodium nucleate was then dialyzed against distilled water in the customary manner and lyophilized.

Method II. The frozen sponge was homogenized with 7.5% sodium chloride solution containing 0.01*M* sodium arsenate (100 ml. of solution for every 100 g. of wet sponge), and the homogenate was allowed to stand overnight at 5°. It was then strained through cheesecloth, diluted with four volumes of water, and stirred gently while cetyltrimethylammonium bromide was added in small amounts until the precipitation of the insoluble quaternary salts was complete. The suspension was allowed to stand at 5° for 24 hr., when the precipitate was collected by centrifugation and thoroughly extracted with 10% sodium chloride solution. Centrifugation of the extract afforded a clear solution which was diluted with two volumes of ethanol and centrifuged. The precipitate was washed successively with 80% ethanol, 95% ethanol, and acetone, and dried *in vacuo* and, if necessary, stored at −20°. It was redissolved in a 10% sodium chloride solution, and deproteinized and further purified over the cetyltrimethylammonium salt as described under Method I.

Method III. The frozen sponge was homogenized for about 3 min. with an approximately equal weight of 10% sodium chloride solution, and the homogenate was allowed to stand at 3° for 24 hr. It was then strained through cheesecloth, and the solid material retained by the cloth was re-extracted twice more with 10% sodium chloride solution. The combined extracts were centrifuged to remove suspended matter and then diluted with two volumes of ethanol. The precipitate was collected by centrifugation, suspended in physiological saline solution (100 ml. for every 100 g. of the original sponge), and the suspension stirred with one-tenth of a volume of a 5% solution of sodium dodecylsulfate in 45% ethanol at 3° for 2 days. Sufficient solid sodium chloride was then added to make the solution molar, and stirring was continued at room temperature for a further 3 hr. The protein precipitate was removed by centrifugation, and the clear solution was diluted with two volumes of ethanol. The precipitate was collected by centrifugation and extracted for 24 hr. at 3° with *M* sodium chloride solution (100 ml. for every 100 g. of the original sponge). Further deproteinization according to Sevag[8] and purification over the cetyltrimethylammonium salt was done as described under Method I.

Method IVa. The sponge was extracted with an approximately equal weight of 10% sodium chloride solution as described under Method III. Two volumes of ethanol were added to the extract, and the resulting precipitate collected by centrifugation and deproteinized according to Sevag's method.[8] The nucleic acid-polysaccharide mixture thus obtained was precipitated three times with ethanol from a *M* sodium chloride solution, washed successively with 75% ethanol, 95% ethanol, and acetone, and then dried under diminished pressure.

Method IVb. The nucleic acid preparation obtained by Method IVa was further deproteinized by shaking its aqueous solution with 90% phenol, followed by centrifugation and separation of the aqueous, phenolic, and protein phases. The aqueous phase was twice more treated with phenol in an analogous manner. After the final treatment the aqueous phase was made molar with respect to sodium chloride, and the nucleic acid-polysaccharide mixture was precipitated with ethanol, and then washed and dried as described under Method IVa.

Degradation of the sponge nucleic acids. Chromatographic techniques and solvent systems. The descending chromatographic technique was employed and Whatman No. 1 paper was used. The chromatograms were analyzed under ultraviolet light. The following solvent systems were used:

(15) M. W. de Laubenfels, Carnegie Institution of Washington, Publication No. 467 (1936).

(16) Technical Grade Quality, supplied by Matheson, Coleman and Bell.

A. 1-Butanol, ethanol, and 5*N* hydrochloric acid in volumes of 3:2:2.[17]

B. 1-Butanol saturated with water at about 23° and 15*N* ammonium hydroxide in volumes of 100:1.[18]

C. Isobutyric acid and 0.5*N* ammonium hydroxide in volumes of 10:6.[19]

D. One volume of pyridine added to the upper layer of a mixture of 1-butanol, pyridine, and water in volumes of 3:1:1.5.[20]

E. 1-Butanol saturated with water at about 23°.

F. 1-Butanol, ethanol, and water in volumes of 4:1:5.[20]

G. 2-Propanol (65 ml.), concentrated hydrochloric acid corresponding to 0.2 g. mol. of HCl, and water to give a total of 100 ml.[21]

H. The upper layer of a mixture of 1-butanol, glacial acetic acid, and water in volumes of 4:1:5.

The nucleotides used as marker substances were mixtures of 2′ and 3′ isomers.

Electrophoresis. The buffer solution used in the electrophoresis of the pentose nucleosides was 0.1*M* sodium borate, *p*H 9.3.[22] Whatman No. 3 mm. paper was used and a gradient of 20 volts/cm. was maintained.

Ultraviolet absorption spectra. These were determined with a Cary Recording Spectrophotometer (Model II S).

Degradation of the sponge ribonucleic acids. (A) Acid hydrolysis. Smith and Markham's[23] method was applied to the nucleic acid preparations which gave a negative or only weakly positive Dische test for deoxypentose nucleic acids. The following quantities of crude nucleic acids were hydrolyzed each with 0.2 ml. of *N* hydrochloric acid in a sealed tube for 1 hr. at 100°: *Halichondria panicea*, 5 mg.; *Verongia fistularis*, 8 mg.; *Spheciospongia othello*, 5 mg.; *Tedania ignis*, 8 m.; *Terpios fugax*, 3 mg.; *Ircinia fasciculata* 4 mg.; and *Tethia actinia*, 5 mg. In each case the hydrolyzate was directly applied to a paper strip and chromatographed using the solvent system A against authentic markers of guanine, adenine, cytidylic acid, and uridylic acid. No differences were noted between the R_f values of the sponge products and the markers. The spots derived from the sponge nucleic acids and appropriate blanks of equal size were cut from the chromatogram, and each was eluted with 5 ml. of 0.1*N* hydrochloric acid. The spectra of the eluates at both *p*H 1 and *p*H 12.5 were identical with those recorded for the corresponding marker substances. Table II lists the molar proportions of degradation products calculated from the optical densities of the original acidic eluates at their respective absorption maxima.

B. Enzymatic hydrolysis. The enzymatic hydrolyses were carried out with the crude venom of the rattle snake, *Crotalus adamanteus*.[24] *B1. Ribonucleic acid-polysaccharide mixtures.* The same nucleic acid preparations were used as in the acid hydrolyses (A), but in approximately double the amounts. A solution of the nucleic acid and 2.3 mg. of the crude snake venom in 1.3 ml. of 0.1*M* ammonium acetate buffer (*p*H 8.5) containing 1.3 mg. of magnesium chloride was incubated for 20 hr. at 37°. Two volumes of 95% ethanol were added, and the resulting precipitate of polysaccharide was removed by centrifugation. In the case of the highly purified nucleic acid preparation from *Halichondria*, no precipitate was formed at this stage, but only a slight turbidity due to the separation of the enzyme. In all cases the centrifuged, clear solutions were concentrated *in vacuo* to about 0.2 ml. Aliquots of these solutions were chromatographed in solvent system B for 40 hr. against authentic guanosine, adenosine, cytidine, and uridine markers. No R_f differences were noted between the authentic and the sponge nucleosides. The identity of the sponge nucleosides with the marker substances was confirmed by measurements of the ultraviolet absorption spectra of the eluted spots in acid, neutral, and alkaline media, and by electrophoretic comparison of the sponge nucleosides with the authentic materials in 0.1*M* sodium borate buffer of *p*H 9.3.

B2. Ribonucleic acid-deoxyribonucleic acid-polysaccharide mixtures. The following method was applied to the nucleic acid preparations from the following sponges, and on the quantities indicated in parentheses: *Dysidea crawshayi* (10 mg.), *Cinachyra cavernosa* (30 mg.), *Ianthella ardis* (20 mg.), and *Leucetta floridana* (10 mg.). The nucleic acid preparation was dissolved in 0.5 ml. of 0.3*N* potassium hydroxide, and the solution was incubated at 37° for 20 hr. The *p*H was then adjusted to 2.5 by addition of 3% perchloric acid solution. Unreacted deoxyribonucleic acid and polysaccharides were precipitated by addition of two volumes of 95% ethanol, collected by centrifugation, washed successively with 80% and 90% ethanol, and stored for the analysis of the deoxyribonucleic acid. The acidic supernatant solution remaining after removal of the deoxyribonucleic acid and the polysaccharides was neutralized with dilute potassium hydroxide and concentrated to about 0.1 ml. Precipitated potassium perchlorate was removed by centrifugation, and the supernatant solution was evaporated to dryness at 30°. The residue was dissolved in 0.3 ml. of 0.1*M* ammonium acetate buffer, *p*H 5.3, the solution centrifuged, and the supernatant liquid was incubated at 37° for 3 hr. with 0.003 ml. of a prostatic phosphatase solution,[25] prepared according to the standard procedure of Schmidt.[26] Two volumes of ethanol were added, the mixture clarified by centrifugation, and the supernatant liquid concentrated to about 0.1–0.2 ml. This material was then transferred onto paper and chromatographed in solvent system E for 2–3 days. The nucleoside spots were identified as described under B1. In some cases the guanosine spot streaked from the origin presumably because of the continued presence of potassium perchlorate. No other differences were observed in the chromatographic, electrophoretic, and ultraviolet spectral characteristics of any of the sponge ribonucleosides and the authentic nucleosides, guanosine, cytidine, uridine, and adenosine.

Degradation of Cryptotethia crypta ribonucleic acid. Fifty mg. of the ribonucleic acid–deoxyribonucleic acid–polysaccharide mixture obtained from *Cryptotethia* by extraction method IVb were hydrolyzed in 2 ml. of 0.3*N* potassium hydroxide for 20 hr. at 37°. The nucleotides obtained from the ribonucleic acid were then separated from the deoxyribonucleic acid and the polysaccharides, and hydrolyzed to the nucleosides according to the method described under B2. The total nucleoside solution was transferred to a paper strip and chromatographed in solvent system E for 4 days. The four bands, corresponding in R_f values to guanosine, cytidine, uridine, and adenosine, were each eluted with 5 ml. of water, and the ultraviolet spectra of the eluates were measured. They were identical with those of the authentic ribonucleosides. Their identity was further confirmed by the respective mobilities in a sodium borate buffer, *p*H 9.3, and their ultraviolet absorption spectra in acid and base. The molecular proportions of the sponge ribonucleosides, estimated from the optical densities of the aqueous eluates at their absorption maxima were guanosine, 20.0; adenosine,

(17) G. E. Eastman, *Biochim. et Biophys. Acta*, **9**, 258 (1952).

(18) W. S. Macnutt, *Biochem. J.*, **50**, 384 (1952).

(19) B. Magasanik, E. Vischer, R. Doniger, D. Elson, and E. Chargaff, *J. Biol. Chem.*, **186**, 37 (1950).

(20) E. Chargaff, E. Vischer, R. Doniger, R. Green, and F. Misani, *J. Biol. Chem.*, **177**, 405 (1949).

(21) G. R. Wyatt, *Biochem. J.*, **48**, 584 (1951).

(22) L. Jaenecke and T. Volbrechthausen, *Naturwiss.*, **39**, 86 (1952).

(23) J. D. Smith and R. Markham, *Biochem. J.*, **46**, 509 (1950).

(24) Obtained from Ross Allen's Reptile Institute.

(25) We are indebted to Dr. C. E. Carter, Yale University, for this preparation.

(26) G. Schmidt, *Methods in Enzymology*, Academic Press, 1955, Vol. II, p. 529.

TABLE III

SPONGE DEOXYRIBONUCLEOSIDE PROPORTIONS

Sponge	Adenine Deoxyriboside	Thymidine	Guanine Deoxyriboside	Cytosine Deoxyriboside	Purines / Pyrimidine
Cryptotethia crypta	10.0	9.0	8.9	8.9	1.05
Dysidia crawshayi	10.0	10.4	7.5	7.0	1.01
Cinachyra cavernosa	10.0	10.0	9.5	9.4	1.01
Ianthella ardis	10.0	10.0	15.3	15.0	1.01
Leucetta floridana	10.0	11.1	5.7	5.6	0.94

10.0; cytidine, 13.1; uridine, 7.0; and purine:pyrimidine, 1.65. The total weight of the nucleosides was approximately 300 μg., corresponding to 0.75% of ribonucleic acid in the original nucleic acid–polysaccharide mixture.

Identification of ribose. Hydrolyses were carried out with *N* hydrochloric acid in the usual manner.[23] The hydrolyzate was neutralized by passage through a column of Dowex-I (OH-form) before application to the paper. The chromatography was carried out in solvent systems D, E, and F, and the spots were detected with aniline hydrogen phthalate spray.[27] Where deoxyribonucleic acid and polysaccharides were present, the ribonucleic acid was first degraded enzymatically to the nucleosides, and adenosine was separated from the mixture by paper chromatography and subsequently eluted and hydrolyzed in the usual manner. The carbohydrate spots obtained from each of the sponge ribonucleic acid preparations by any one of the methods showed no differences in R_f value from authentic D-ribose.

Degradation of sponge deoxyribonucleic acids. A. Deoxyribonucleosides. Deoxyribonucleic acid–polysaccharide mixtures obtained by the method described under B2 were further freed from residual potassium perchlorate by two ethanol precipitations from *M* sodium chloride solution. The dried preparation was then dissolved in 0.3 ml. of 0.1*M* ammonium acetate buffer, *p*H 6.8, containing 300 μg. of magnesium chloride and about 50 μg. of deoxyribonuclease, and the solution was incubated at 37° for 5 hr. About 2 mg. of crude snake venom was then added, and the incubation continued for 15 hr. The mixture was diluted with two volumes of ethanol, and the polysaccharide precipitate removed by centrifugation. The supernatant solution was concentrated to about 0.1 ml., and chromatographed in solvent system E against the authentic deoxyribonucleosides of guanine, adenine, cytosine, and thymine. The four spots given by the sponge nucleosides showed the same R_f values as the authentic markers.

In the case of *Cryptotethia crypta*, the cytosine and thymine nucleosides were eluted from a duplicate chromatogram, and rechromatographed in three additional solvent systems, B, G, and H. In these solvent systems they also gave the same R_f values as authentic cytosine deoxyriboside and thymidine, respectively. Spongothymidine gives an appreciably lower R_f value than thymidine in all four solvent systems.

In all cases the nucleoside spots were cut from the chromatograms together with blanks of equal size, each eluted with 5 ml. of water for 24 hr., and the ultraviolet absorption spectra of the eluates were measured. The spectra in neutral, acidic, and basic solutions did not differ from those of the corresponding authentic nucleosides. Table III lists the molar proportions of the individual deoxyribonucleosides as calculated from the absorption maxima of the original neutral eluates.

Degradation of sponge deoxyribonucleic acids. B. Free bases. Deoxyribonucleic acid–polysaccharide mixtures, such as used in the degradation to the nucleosides, were hydrolyzed with 98% formic acid in sealed tubes for 30 min. at 175°.[21] The formic acid was removed *in vacuo*, and the residues dissolved in 0.1*N* hydrochloric acid and chromatographed in solvent system G against guanine, adenine, cytosine, and thymine markers. The chromatograms were marred in most cases by streaking, fluorescent polysaccharide degradation products, but dark absorbing spots with R_f values equal to those of the markers were readily discernable. In no case were any other spots observed.

Identification of 2-deoxy-D-ribose. Polysaccharide-free solutions of sponge deoxyribonucleosides, prepared as described above (A) were concentrated to about 0.1 ml. and hydrolyzed by bringing the solutions to *p*H 1 with hydrochloric acid, and by heating them in sealed tubes at 100° for 10 min. The hydrolyzates were applied directly to paper and chromatographed in solvent systems D, E, and F against authentic 2-deoxy-D-ribose. Spots were detected with *m*-phenylenediamine spray.[20] No R_f differences between the sugar from the sponge nucleic acid and authentic 2-deoxy-D-ribose could be detected. In the case of *Cryptotethia crypta*, adeninedeoxyriboside and guaninedeoxyriboside, following chromatographic separation and elution, were individually hydrolyzed. They gave 2-deoxy-D-ribose as the only carbohydrate moiety.

(27) S. M. Partridge, *Nature*, **164**, 443 (1949).

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