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Contributions to the Study of Marine Products. XL. The Nucleosides of Sponges.[1] IV. Spongosine[2]

WERNER BERGMANN AND DEREK C. BURKE

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Spongosine, one of the nucleosides from the sponge *Cryptotethia crypta,* has been shown to be the D-riboside of 2-methoxy-6-aminopurine (2-methoxyadenine).

A few years ago the senior author and Feeney[3] reported the isolation from the Carribean sponge, *Cryptotethia crypta,* of a mixture of nucleosides of a type not previously encountered in nature. Two of these, spongothymidine and spongouridine, have since been shown by the present authors[4] to be the 3-β-D-arabofuranosides of thymine and uracil respectively. By means of chromatographic methods, recently described,[4] it has now been possible to obtain a third nucleoside, spongosine, in quantities permitting the identification of its components. As shown previously,[3] spongosine is the pentoside of an amino-oxypurine with an additional CH_2-unit, presumably in form of a methyl group.

When oxidized with sodium periodate spongosine consumes one mole of the reagent without formation of formic acid.[5] The oxidation proceeds very rapidly and is completed within five minutes. This observation proves spongosine to be a pentofuranoside with *cis*-hydroxyl groups at C_2-C_3. It also differentiates spongosine from the two other sponge-nucleosides which have been shown to be arabinosides, *i.e.*, with *trans*-hydroxyls at C_2-C_3. In accordance with this difference, paper ionophoresis of spongosine in a phosphate buffer[6] shows a relatively high Mg value (0.56).

Since it is well known that purine nucleosides are more readily hydrolysed than are their pyrimidine analogs, it is not surprising that spongosine is easily split into the purine and carbohydrate moieties under conditions which leave the other sponge nucleosides unaffected. The separation of the two fragments is best carried out on mixed Dowex columns. The pentose moiety was not isolated in a crystalline state, but was proven to be D-ribose by means of paper chromatography, paper ionophoresis in a borate buffer,[6] preparation of the phenylosazone, and the comparison of its physical properties with those of authentic material.

In the first paper of this series[3] it was revealed that the purine derived from spongosine, $C_6H_7N_5O$, possesses one carbon atom more than accounted for by the purine ring system. The possibility that this carbon atom might be part of a ring system,

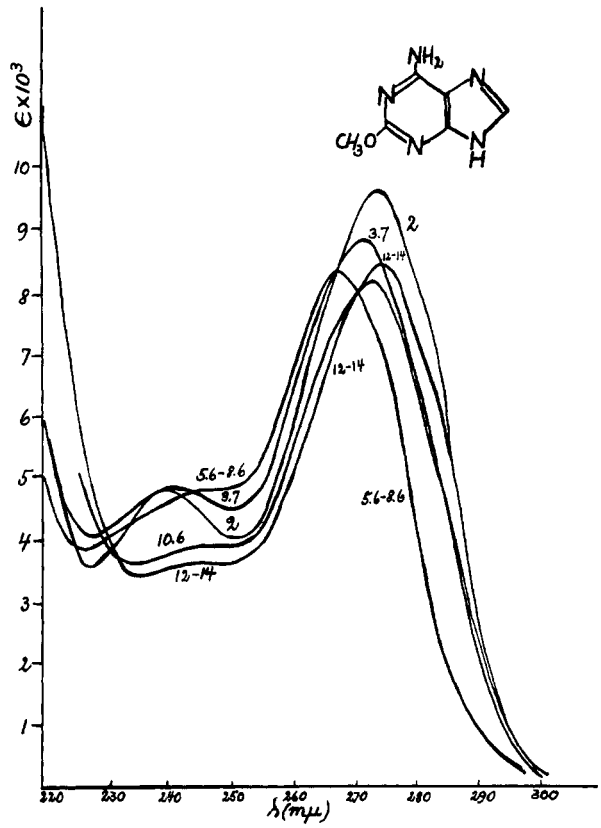

FIG. 1.—VARIATION OF THE SPECTRUM OF 2-METHOXYADENINE WITH *p*H.

such as in a pteridine, was contraindicated by the ultraviolet absorption spectrum. The spectra of the purine taken at different *p*H values (Figure 1) show two *p*K values of 3.7 and 10.0; under the same conditions spongosine (Figure 2) shows only one, at 3.2. The *p*K values of 3.2 and 3.7 respectively appear to be associated with the presence of an amino group which previously had been indicated through the action of nitrous acid on the purine.[3]

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(2) The authors are greatly indebted to Mr. M. Bishop of the Lerner Marine Laboratory, Bimini, Bahamas, for his valuable help in obtaining sponge material.

(3) Bergmann and Feeney, *J. Org. Chem.,* 16, 981 (1951).

(4) Bergmann and Burke, *J. Org. Chem.,* 20, 1501 (1955).

(5) Lythgoe and Todd, *J. Chem. Soc.,* 592 (1944).

(6) Burke, *Chem. and Industry,* 1510 (1954).

The pK value of 10.0 is attributable to the acidic hydrogen of the imidazole ring.[7] Since adenine is the only other naturally occurring purine showing similar spectral behavior, the conclusion was drawn that the new purine also carried an amino group at C-6.

The ultraviolet absorption spectra of spongosine (Figure 2) and its derived base (Figure 1) are unusual because they show two absorption peaks in acid, but only one in alkaline solution. Two similar maxima in acid solution are also shown by the

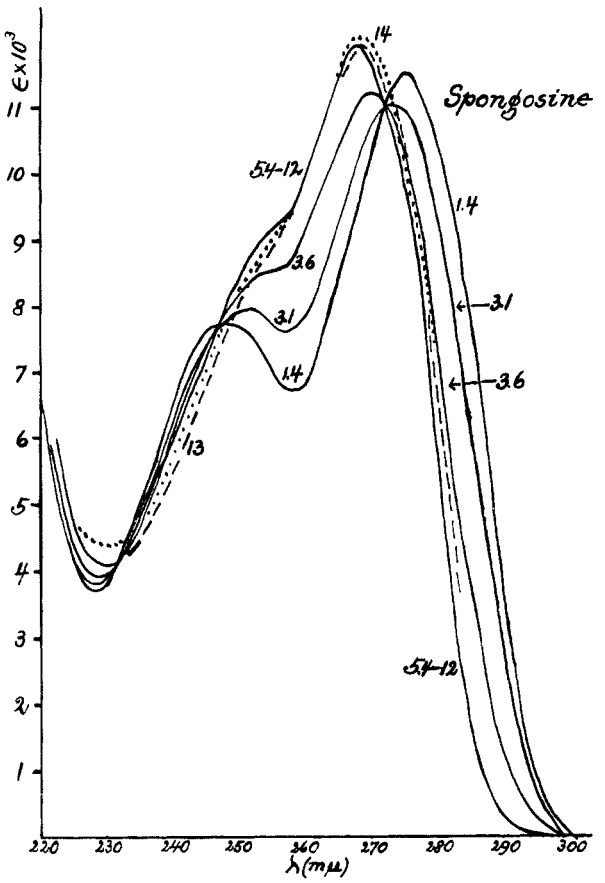

FIG. 2.—VARIATION OF THE SPECTRUM OF SPONGOSINE WITH pH..

spectra of crotonoside,[8] the riboside of 2-oxy-6-aminopurine. These spectra, however, show two additional peaks in alkaline solution which have been attributed to an ionizable group at the 2-position of the purine ring system.[9] Since spongosine shows but a single peak in this region the presence in the 2-position of either hydroxyl or amino group is contraindicated.

Incubation of spongosine with a sample of intestinal phosphatase containing substantial amounts of adenosine deaminase[10] led to a rapid and complete deamination. Since the action of this enzyme is held to be specific for 6-aminopurines, it must be assumed that the aminogroup of spongosine is indeed in the 6-position.

There remained the problem of assigning positions to the methyl group and the oxygen. For reasons given above attachment of the latter to C-2 in form of an ionizable group seemed quite unlikely. Its presence at C-8 appeared equally improbable because of the differences between the ultraviolet spectra of the new purine (Figure 1) and its deaminated derivative (Figure 3) and those of 6-amino-8-hydroxy- and 6,8-dihydroxypurine.[11]

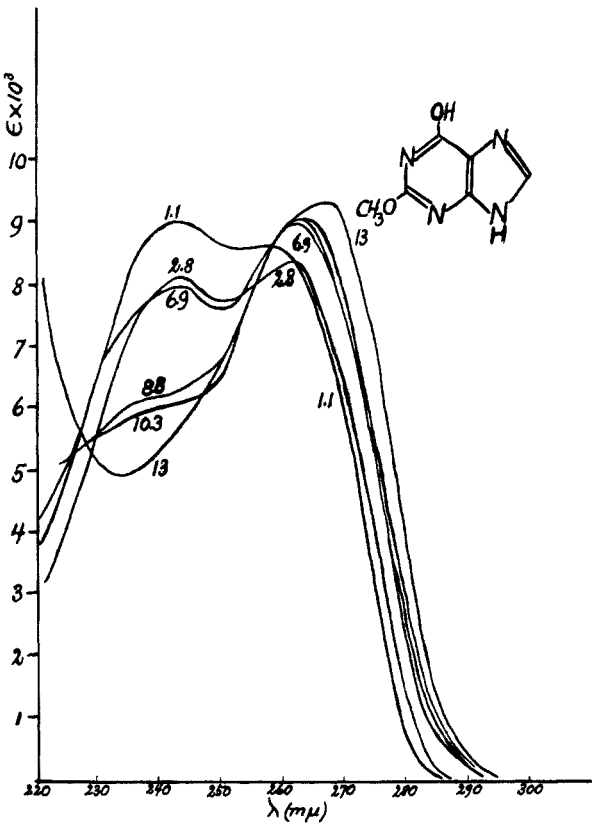

FIG. 3.—VARIATION OF THE SPECTRUM OF 2-METHOXY-6-OXYPURINE WITH pH.

The possibility then was considered that the two unplaced units were combined in form of a methoxyl group. A Zeisel methoxyl determination showed that this is indeed the case and that the purine is a methoxy-adenine. For reasons given above C-2 appeared to be the most likely location of the methoxyl group. In addition, the purine gives a positive reaction with the Folin phenol reagent which is regarded as specific for 2-substituted purines.[12] 2-Methoxyadenine, which had not previously been described, then was synthesized by heat-

(7) Taylor, *J. Chem. Soc.*, 765 (1948).
(8) Davoll, *J. Am. Chem. Soc.*, **73**, 3174 (1951).
(9) Cavalieri, Bendich, Tinker, and Brown, *J. Am. Chem. Soc.*, **70**, 3875 (1948).
(10) Obtained from Pentex Co.; see Kalkar, *J. Biol. Chem.*, **167**, 461 (1947).
(11) Cavalieri and Bendich, *J. Am. Chem. Soc.*, **72**, 2587 (1950).
(12) Hitchings, *J. Biol. Chem.*, **139**, 843 (1941).

ing 2-chloroadenine[13] in a sealed tube with sodium methoxide in methanol. After purification on an ion-exchange column, the synthetic product proved to be identical in all respects with the compound derived from spongosine.

Spongosine is therefore a ribofuranosyl-2-methoxy-6-aminopurine or 2-O-methylcrotonoside. The point and configuration of the junction of the two moieties will be definitely established by a synthesis which is to be the subject of a later publication. It is of interest to note that spongosine, a purine nucleoside, belongs to well-known riboside type while the pyrimidine nucleosides from the sponge are of the new arabinoside type. Work now in progress in this laboratory aims to ascertain whether both types of nucleosides are present in the sponge nucleic acid. The base derived from spongosine seems to be the first methoxypurine to be found in nature. In addition, it should be pointed out that the methoxyl group, so common among plant products, appears to be quite rare in the animal world. In fact, as far as the authors are aware, spongosine represents the first example of the occurrence of a methoxyl compound among animals.

EXPERIMENTAL

Spongosine. The nucleoside was separated from the original mixture by means of an ion-exchange column as previously described.[4] After several recrystallizations from hot water, it melted at 193°.[3]

Anal. Calc'd for $C_{11}H_{15}N_5O_5$: OCH_3, 10.44. Found: OCH_3, 10.49.

When oxidized with sodium periodate according to the standard procedure,[4] 0.96 mole of oxidant was consumed during the first 5 minutes and 1.1 moles after 96 hours.

Hydrolysis of spongosine and identification of the ribose. The nucleoside (203 mg.) was refluxed with 0.1 *N* sulfuric acid (10 ml.) for 90 minutes. After cooling, ammonium hydroxide was added to the solution, the excess base was removed by heating, and the precipitate was collected. Paper chromatography showed it to consist of the purine and some unreacted material.

The filtrate was passed over a column (25 cm. × 2 cm.²) of a mixture of Dowex 1 and Dowex 50. A solution was obtained which showed no selective absorption in ultraviolet light. Evaporation of the solvent afforded a syrup, $[\alpha]_D^{21}$ −6°, which was shown to be ribose by paper chromatography in *n*-butanol saturated with water[14] and with the upper layer of a *n*-butanol-water-ethanol mixture (5:4:1).[15] For further identification the sugar was converted to the phenylosazone. After recrystallization from aqueous methanol it melted at 154–155° and the same melting point was obtained when the substance was mixed with authentic ribose phenylosazone. The infrared spectra of the two samples were also found to be identical and different from that of D-xylose phenylosazone.

The nucleoside (5 mg.) was incubated in a veronal buffer[16] with a trace of magnesium chloride and 1 mg. of intestinal phosphatase for 24 hours at 37°. After this treatment spongosine was no longer detectable by paper ionophoresis[6] and paper chromatography in several solvents.

2-Methoxyadenine. 2-Chloroadenine (1 g.)[13] was heated in a sealed tube for 3 hours at 150° with a sodium methoxide solution prepared from sodium (3 g.) and anhydrous methanol (60 ml.). Water (100 ml.) then was added and the mixture was poured on a Dowex 1 ion-exchange column (50 cm. × 10 cm.²; flow rate 1.5 ml./min.). Elution with ammonium formate-ammonium hydroxide buffer of *p*H 8.4 first gave a small amount of an unidentified material and then the 2-methoxyadenine; m.p. 275° (dec.).

Anal. Calc'd for $C_6H_7N_5O$: C, 43.6; H, 4.3; N, 42.4. Found: C, 43.45; H, 4.32; N, 42.2.

When mixed with the purine derived from spongosine[3] no depression of the melting point was observed. The two compounds showed identical infrared spectra in Nujol, identical ultraviolet spectra at *p*H 1, 7, and 12, identical mobility in paper electrophoresis at *p*H 2.3, 3.5, and 10.3, and the same R_f values in the following solvents or solvent mixtures: *n*-butanol saturated with ammonia; water (4:1), *n*-butanol:ethanol:water (5:1:4), and *n*-butanol:diethylene glycol:water (4:1:1).[17]

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(13) Davoll and Lowry, *J. Am. Chem. Soc.*, **72**, 1564 (1950).

(14) Zamenhof, Leidy, Fitzgerald, Alexander, and Chargaff, *J. Biol. Chem.*, **203**, 695 (1953).

(15) Hunt and Jones, *Discussions Faraday Soc.*, No. 7, 269 (1949).

(16) Michaelis, *J. Biol. Chem.*, **87**, 33 (1930).

(17) Vischer and Chargaff, *J. Biol. Chem.*, **176**, 703 (1948).