Manganese-dioxide oxidation of the synthetic 11-mono-*cis* isomer yielded the corresponding aldehyde. The product of this reaction was identified as neoretinene b by Prof. George Wald[10] through its condensation with opsin to yield rhodopsin.

(10) Biological Laboratories, Harvard University, Cambridge, Mass.

ORGANIC CHEMISTRY DIVISION
ORTHO RESEARCH FOUNDATION WILLIAM OROSHNIK
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RECEIVED MARCH 23, 1956

A NEW INOSAMINE FROM AN ANTIBIOTIC

Sir:

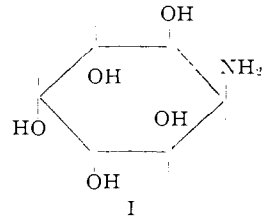
The occurrence of non-synthetic amino analogs of inositol ("inosamines")[1] has been reported only in the case of the streptomycin[2] and neomycin[3] families of antibiotics; in each case the compound was a diamine.

We have isolated a *mono*-inosamine having a configuration unprecedented in natural inositols. By hydrolysis of a new antibiotic (designated in these laboratories as 1703–18B[4]) with concentrated hydrochloric acid, we obtained a compound, m.p. 217–221[6] (dec.) (*Anal.* Calcd. for $C_6H_{13}NO_5 \cdot HCl$ (215.6): C, 33.42; H, 6.54; N, 6.50; Cl, 16.44. Found: C, 33.54; H, 6.83; N (Dumas), 6.20; N (Van Slyke), 6.52; Cl, 16.37) which yielded a free base, m.p. 238–240° (dec.) (*Anal.* Calcd. for $C_6H_{13}NO_5$ (179.2): C, 40.22; H, 7.31; N, 7.82. Found: C, 40.40; H, 7.30; N, 7.87).

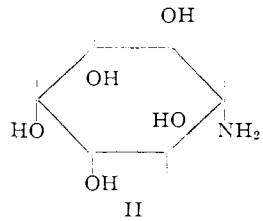
The base consumed 6.14 moles of periodate (inositol, 6.20) with no formaldehyde formation. The optical rotation of the base in water or in aqueous ammonium molybdate[7] was zero; the hexa-acetate, m.p. 277.5–278.5° (*Anal.* Calcd. for $C_{18}H_{25}NO_{11}$ (431.4): C, 50.11; H, 5.84; N, 3.25; acetyl, 59.85. Found: C, 49.88; H, 6.00; N, 3.32; acetyl 58.61) in chloroform was also optically inactive, and the inactive base was recovered from its nicely crystalline salt (m.p. 230–232°) with *d*-camphorsulfonic acid.

The above data show the compound to be a meso-inosamine. Three of the eight possible meso-inosamines have been reported[1,8,9]; the physical properties of the new inosamine do not correspond to those of any of them.

Under the conditions described by Angyal and MacDonald[10] the phthalimido derivative,[11] m.p. 255–261° (dec.) (*Anal.* Calcd. for $C_{14}H_{15}NO_7$ (309.3): C, 54.37; H, 4.89; N, 4.53. Found: C, 54.03; H, 5.08; N, 4.86) of the inosamine furnished a single, racemic *mono*-acetonide, m.p. 210–212° (*Anal.* Calcd. for $C_{17}H_{19}NO_7$: C, 58.45; H, 5.48; N, 4.01. Found: C, 58.23; H, 5.84; N, 3.92) which took up one mole of periodate. Only two structures, I and II, are thus possible for the inosamine.

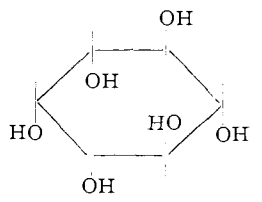


I

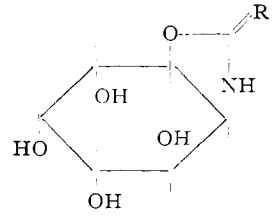


II

Nitrous acid deamination of the inosamine produced the known "meso" inositol III. In the inosamine series this reaction is known to proceed with inversion[12]; therefore, our compound is I.



III



IV, a, R = O
b, R = S

Reaction of the inosamine with carbobenzyloxy chloride provides further evidence for structure I; under appropriate conditions the chief product is the cyclic carbamate IVa, m.p. 203–205° (*Anal.* Calcd. for $C_7H_{11}NO_6$ (205.2): C, 40.97; H, 5.40; N, 6.83. Found: C, 41.20; H, 5.56; N, 6.81). The carbamate consumed 2.92 moles of periodate (mannitol standard, 5.00), as required for structure IVa. Similarly, the thiocarbamate IVb, m.p. 245° (dec.) is produced by reaction of I with phenyl isothiocyanate.[8b,13] (*Anal.* Calcd. for $C_7H_{11}NSO_5$ (221.2): C, 37.99; H, 5.01; N, 6.33; S, 14.49. Found: C, 38.16; H, 5.23; N, 6.37; S, 14.68).

We believe that this constitutes the first occurrence of a mono-inosamine and of a cyclitol of this configuration. The structure of antibiotic 1703–18B will be the subject of a future publication.

(12) (a) T. Posternak, *Helv. Chim. Acta*, **33**, 1597 (1950); (b) H. Straube-Rieke, H. A. Lardy and L. Anderson, THIS JOURNAL, **75**, 694 (1953).

(13) M. Roux, *Ann. Chim.*, [8] **1**, 112 (1904). The question of tautomeric forms is immaterial to our argument.

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RECEIVED MAY 2, 1956

IDENTIFICATION OF ENZYMATICALLY ACTIVE SULFATE AS ADENOSINE-3'-PHOSPHATE-5'-PHOSPHOSULFATE[1]

Sir:

DeMeio's pioneering work has shown that enzymatic sulfate activation is an ATP-linked reac-

(1) This investigation was supported by research grants from the Cancer Institute of the National Institutes of Health, Public Health Service, and the Life Insurance Medical Research Fund.

(1) H. E. Carter, R. K. Clarke, B. Lytle and G. E. McCasland, *J. Biol. Chem.*, **175**, 683 (1943).

(2) (a) H. E. Carter, *et al.*, *Science*, **103**, 53 (1946); (b) J. Fried, A. Boyak and O. Wintersteiner, *J. Biol. Chem.*, **162**, 393 (1946); (c) R. L. Peck, *et al.*, THIS JOURNAL, **68**, 776 (1946).

(3) F. A. Kuehl, M. N. Bishop and K. Folkers, *ibid.*, **73**, 881 (1951).

(4) On the basis of published data[5] it appears that this antibiotic is similar to hygromycin, although not identical with it.

(5) R. L. Mann, R. M. Gale and F. R. van Abeele, *Antibiotics and Chemotherapy*, **3**, 1279 (1953).

(6) All melting points were determined on the Kofler hot stage and are corrected.

(7) W. W. Pigman and R. M. Goepp, Jr., "Chemistry of the Carbohydrates," Academic Press, New York, N. Y., 1948, p. 248.

(8) (a) L. Anderson and H. A. Lardy, THIS JOURNAL, **72**, 3141 (1950); (b) G. E. McCasland, *ibid.*, **73**, 2295 (1951).

(9) (a) J. M. Grosheintz and H. O. L. Fischer, *ibid.*, **70**, 1479 (1948); (b) H. D. Orloff, *Chem. Revs.*, **54**, 347 (1954).

(10) S. J. Angyal and C. G. MacDonald, *J. Chem. Soc.*, 686 (1952).

(11) We are indebted to Dr. B. R. Baker for suggesting this derivative for the purpose.

tion.[2] More recently, Hilz and Lipmann[3] preliminarily identified active sulfate chromatographically as an adenosine derivative. We wish to report now that active sulfate has been characterized as adenosine-3′-phosphate-5′-phosphosulfate (PAPS). In our system PAPS derives from adenosine-5′-triphosphate + sulfate and its formation must include phosphorylation in 3′-position.

PAPS is assayed by use of the sulfate–nitrophenol transfer enzyme.[2,4,5] For the preparation of the compound, a liver enzyme prepared by a modification of the previously described procedure[3] was used. After heat removal of protein, the solution containing an average of 50 μM. of PAPS was applied to a column of Dowex-1 and all nucleotides except PAPS were removed by elution with 4 N formic acid and 0.3 N ammonium formate.[6] PAPS was then eluted with 5 N formic acid and 1 N ammonium formate. After lyophilization overnight the eluate showed on paper electrophoresis[3] two ultraviolet absorbing spots, the faster moving corresponding to active sulfate as localized earlier. The slower moving substance, located midway between ADP and ATP, was identified as 3′,5′-diphosphoadenosine (PAP). The phosphosulfate link is hydrolyzed completely by 0.1 N hydrochloric acid at 37° in 30 minutes. Therefore, the formic acid procedure causes more or less hydrolysis.

As shown in Table I, the analysis of the eluates gave consistently a ratio of adenosine to phosphate of 1:2, independent of the amount of active sulfate

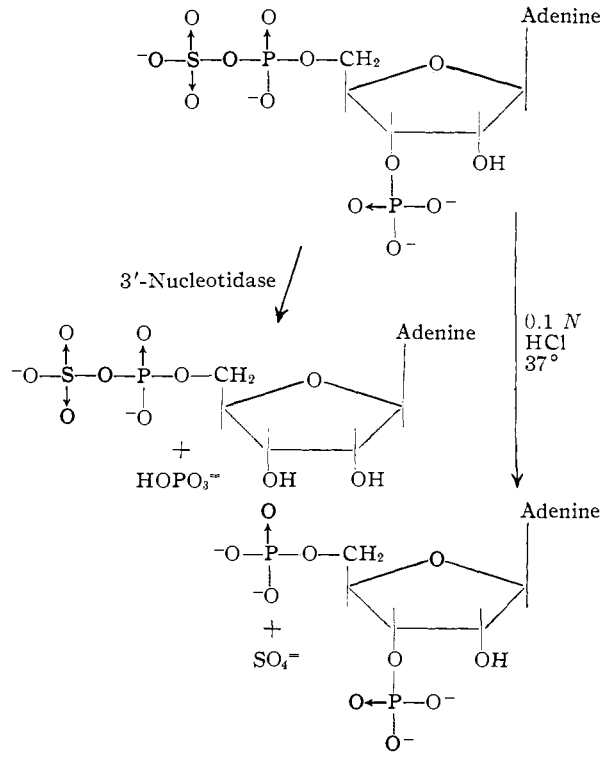
TABLE I

ACTIVE SULFATE FRACTION, ANALYTICAL DATA

Adenosine was determined by absorption at 260 mμ, ribose by the orcinol procedure, and phosphate by the method of Fiske and SubbaRow. Total phosphate was determined after ashing with sulfuric acid. The 12 and 30 minute phosphate was determined by hydrolysis in 1 N HCl at 100°. Phosphate hydrolyzable by the 3′-nucleotidase was determined by the method of Kaplan.[8] We are indebted to Dr. Kaplan for a sample of this enzyme.

Adenosine		1
Ribose		0.95
Phosphate	Total	1.98
	12 minutes	0.53
	30 minutes	1.04
	3′-nucleotidase	0.85
Sulfate, enzymatic		0.2–0.85

present. Acid hydrolysis indicated a phosphate in 2′- or 3′-position on the ribose rather than a pyrophosphate. This was confirmed by the absence of the periodate reaction given by the adenosine-5′-polyphosphates.[7] The second phosphate was eventually further identified as located in 3′-position using Kaplan's specific 3′-nucleotidase.[8] Hydrolysis of S^{35}-marked PAPS with 3′-nucleotidase results in the appearance in the electrochromatogram of a new radioactive spot slightly above ADP which corresponds to adenosine-5′-phosphosulfate (APS); this compound is enzymatically inactive. The degradation of PAPS is formulated as follows:



The position of the sulfate was verified by titration. Hydrolysis of a phosphosulfate should liberate an equivalent of secondary phosphate. For example, mild acid treatment of a preparation containing approximately 5 μM. PAPS gave an increase in titrable material between pH 5 and 8, of 6.0 μM. Further identification was obtained through the confirmation of an earlier suspected reversibility of the reaction: PAPS + nitrophenol $\rightleftarrows$ PAP + nitrophenyl sulfate. The reverse reaction was used for the identification of the desulfo residue. In this manner, 3′,5′-diphosphoadenosine was detected in some commercial preparations of ADP. The sulfate acceptor also was obtained by hydrolysis of coenzyme A, known to contain 3′,5′-diphosphoadenosine.[8]

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RECEIVED APRIL 13, 1956

(9) Fellow of the National Foundation for Infantile Paralysis.

DEUTERIUM ISOTOPE RATE EFFECTS AND STERIC INHIBITION OF HYPERCONJUGATION

Sir:

Recently some deuterium isotope effects on the rate of solvolysis of 2,4,4-trimethyl-2-chloropentane (I) have been observed in this laboratory. These results have an important bearing on the following two problems of current interest in reaction mechanism theory: (1) the cause of such isotope rate

(2) R. H. DeMeio, M. Wizerkaniuk and E. Fabriani, *J. Biol. Chem.*, **203**, 257 (1953).

(3) H. Hilz and F. Lipmann, *Proc. Natl. Acad. Sci.*, **41**, 880 (1955).

(4) S. Bernstein and R. W. McGilvery, *J. Biol. Chem.*, **199**, 745 (1952).

(5) H. L. Segal, *ibid.*, **213**, 161 (1955).

(6) P. Siekevitz and V. R. Potter, *ibid.*, **215**, 221 (1955).

(7) J. G. Buchanan, C. A. Dekker and A. G. Long, *J. Chem. Soc.*, 3162 (1950).

(8) T. P. Wang, L. Shuster and N. O. Kaplan, *J. Biol. Chem.*, **206**, 299 (1954).