crystalline material was dissolved in water and injected into brainless pupae of the silkworm, *Samia cynthia*. In this decisive test for ecdysone, the pupal diapause was terminated and adult development initiated 3 days after the injection of 5 μg into the 2-g brainless pupae.

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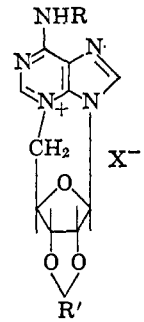
A New "Anhydronucleoside Method"[1] for the Synthesis of Dinucleoside Phosphates

Sir:

Recently the use of anhydronucleosides in the synthesis of the nucleotides and oligonucleotides was reported independently from several laboratories.[2-9]



I, R′ = >C(CH$_3$)$_2$, R = H; Ia, R = COCH$_3$

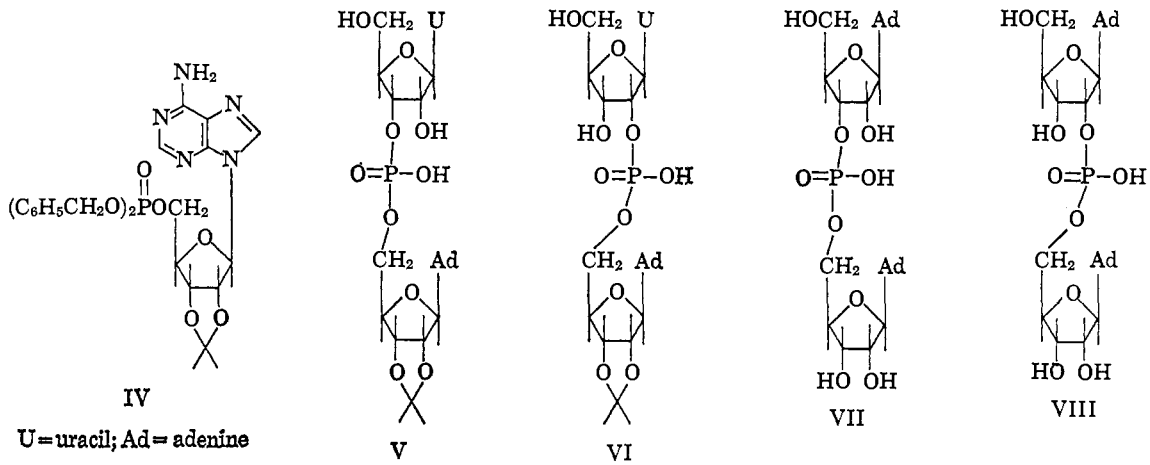
II, R′ = >P(O)–OH, R = H; IIa, R = COCH$_3$

III, R′ = *p*-dimethylamino benzylidene, R = H; IIIa, R = COCH$_3$

So far investigators of the "anhydronucleoside method" have concentrated on the use of pyrimidine anhydronucleosides as starting materials. However, it was anticipated that the anhydronucleosides of the purine series (*viz.*, I,[7] II,[8] and III) might be promising substrates for the oligonucleotide synthesis. Pertinent to the present discussion is Jahn's finding[9] that an acylamino group at position 6 or 1 of purine prohibits or hinders the internal alkylation of 5′-O-tosyladenosines. This finding suggested that acetylation of I to Ia might labilize the nitrogen–carbon bond between N-3 and C-5′ and make C-5′ more vulnerable to the attack by a nucleophile such as a phosphate anion. This has been found to be the case and has led to an extention of the "anhydronucleoside method" to the purine series.

Treatment of I[7] (λ_{max}^{MeOH} 272 mμ) with a twofold excess of acetic anhydride in pyridine at reflux afforded presumably Ia[9] (λ_{max}^{MeOH} 280 mμ). Without isolation of the product Ia the reaction mixture was treated with an equivalent amount of dibenzyl hydrogen phosphate[10] at reflux. After 3 hr the starting material had almost disappeared, with a concomitant appearance of a new spot (corresponding to IV) at R_f 0.78 on the paper chromatograms.[11] The product was isolated by preparative paper chromatography. Assuming an ϵ_{max} of 10,000 for the product, the yield as estimated spectrophotometrically was 89%. Compound IV was crystallized from a mixture of ethyl alcohol and ether, mp 97–98° (lit.[12] 97–98°). The yield of the crystalline product IV was 65%. *Anal.* Calcd for $C_{27}H_{30}O_7N_5P$: C, 57.04; H, 5.29; N, 12.34; P, 5.46. Found: C, 57.05; H, 5.30; N, 12.32; P, 5.21.

Similarly, Ia was treated with an equivalent amount of uridine 2′(3′)-phosphate (diammonium salt) at reflux for 3 hr. A product was purified by preparative paper chromatography[11] and shown to be a 1:1 mixture of uridylyl(3′–5′)-2′,3′-O-isopropylideneadenosine (V) and presumably the isomeric (2′–5′)-dinucleoside phosphate VI (pancreatic ribonuclease resistant) by the ribonuclease assay.[13] Assuming an ϵ_{max} of 23,000 for



IV

U = uracil; Ad = adenine

V

VI

VII

VIII

(1) "Anhydronucleoside method" is tentatively referred to as the synthetic method for the preparation of the nucleotides and oligonucleotides by use of anhydronucleosides as key intermediates.[2-5]

(2) (a) Y. Mizuno, T. Sasaki, T. Kanai, and H. Igarashi, *J. Org. Chem.*, **30**, 1533 (1965); (b) Y. Mizuno, and T. Sasaki, *Tetrahedron Letters*, 4579 (1965).

(3) J. Zemlicka and J. Smrt, *ibid.*, 2081 (1964).

(4) J. Nagyvary and J. S. Roth, *ibid.*, 617 (1965).

(5) K. L. Agarwald and M. M. Dhar, *ibid.*, 2451 (1965).

(6) Also see J. Nagyvary, Abstracts, 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965, p 87C.

(7) V. M. Clark, A. R. Todd, and J. Zussman, *J. Chem. Soc.*, 2952 (1951).

(8) A. M. Michelson, *ibid.*, 1371 (1959).

(9) W. Jahn, *Chem. Ber.*, **98**, 1705 (1965).

(10) V. M. Clark and A. R. Todd, *J. Chem. Soc.*, 2023 (1950).

(11) Paper chromatography was carried out by the use of the descending technique (Toyo Roshi 51A; isopropyl alcohol–concentrated ammonium hydroxide–water, 7:1:2.

(12) J. Baddiley and A. R. Todd, *J. Chem. Soc.*, 648 (1947).

(13) (a) D. M. Brown, D. I. McGrath, and A. R. Todd, *ibid.*, 2708 (1952); (b) D. M. Brown, E. A. Deckker, and A. R. Todd, *ibid.*, 2715 (1952).

V or VI, the yield of the dinucleoside phosphate was 73% on the basis of the uridylic acid employed. Absorption peaks appeared at $\lambda_{max}^{pH\ 5.5}$ 259 mμ; $\lambda_{max}^{pH\ 3.1}$ 259 mμ (ε (p) 23,000).[14]

For the synthesis of adenylyladenosines VII or VIII the reaction of IIIa with adenosine 3′-phosphate was carried out in a manner analogous to that described above. The deblocked product[15] was homogeneous by paper chromatography: R_f in ethyl alcohol–1 *N* ammonium acetate (5:2) 0.22, R_f in *t*-amyl alcohol–formic acid–water (3:2:1) 0.15.[16] A spleen phosphodiesterase treatment under standard conditions[17] gave an equimolar mixture of adenosine 5′-phosphate and adenosine and undegraded dinucleoside phosphate (presumably adenylyl(2′-5′)adenosine (VIII)).[8] Yield of the isomeric diadenosine phosphates was 36%, $\lambda_{max}^{pH\ 7.1}$ 260 mμ (ε (p) 25,500).

(14) ε (p) = A/Cd, where A = absorbance, C = gram-atoms of phosphorus, and d = internal cell length in centimeters.

(15) The 2′,3′-O-blocking group was removed by treatment of VIIb with 0.1 *N* acetic acid for 1 hr at room temperature.

(16) Chromatographic behaviors of our sample (VII) were identical with those reported of an isomeric mixture of adenylyladenosines prepared by Michelson.[8]

(17) W. E. Razzel and H. G. Khorana, *J. Biol. Chem.*, **236**, 1144 (1961).

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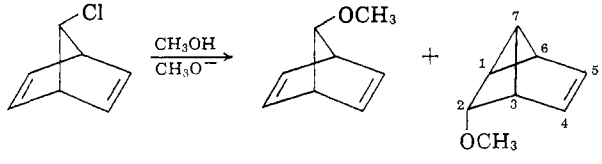
Received November 27, 1965

Methanolysis of 7-Chloronorbornadiene under Alkaline Conditions. Evidence for the Formation of a Labile Tricyclic Intermediate

Sir:

The methanolysis of 7-chloronorbornadiene (I) in the absence of alkali proceeds quantitatively to the formation of 7-methoxynorbornadiene (II).[1] We wish to report that in the presence of an equivalent amount of sodium methoxide the solvolysis produces only 20% of the expected II together with another, highly labile species which is almost instantaneously converted by dilute acid into II. The characteristics of the labile species indicate that it is the hitherto unknown tricyclic derivative III. Consequently, it appears that nucleophiles other than borohydride[2,3] are capable of trapping the intermediate carbonium ion to yield tri-



I II III

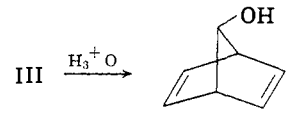
cyclic derivatives. This reaction appears highly promising, therefore, as a simple, convenient synthetic route to functional derivatives of the tricyclic structure, compounds which have not been available previously.

(1) G. Wittig and J. Otten, *Tetrahedron Letters*, No. 10, 601 (1963), reported 68% yield. On reinvestigation, we found quantitative formation of II.

(2) H. C. Brown and H. M. Bell, *J. Am. Chem. Soc.*, **85**, 2324 (1963).

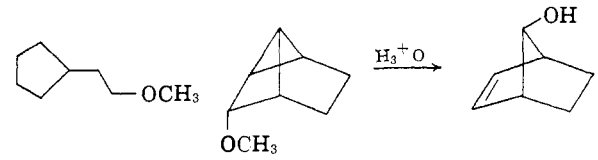
(3) S. Winstein, A. H. Lewin, and K. C. Pande, *ibid.*, **85**, 2324 (1963).

The methanolysis of I in methanol–dioxane (5:1) solution proceeded with a rate of 1.21×10^{-4} sec^{-1} at 16.8°. The same reaction in the presence of an equivalent amount of sodium methoxide gave a rate of 1.67×10^{-4} sec^{-1},[4] and the reaction mixture indicated the formation of a brown polymer beyond 80% reaction. Vapor phase chromatographic analysis showed only 20% yield of II at infinite time. However, when the reaction mixture was treated with dilute but sufficient perchloric acid to quench the action of sodium methoxide (a controlled experiment showed that this treatment quantitatively converts I into 7-norbornadienol (IV), but has no effect on II), the yield of IV indicated by vpc was larger than that of remaining I which was calculated by the rate constant. This excess, which approached maximum at about one half-life and then gradually decreased, strongly suggests the presence of a labile intermediate whose treatment with acid furnishes IV.



III IV

The methanolysis mixture with methoxide at one half-life was reduced at 0° over PtO_2 with about 2 mole equiv of hydrogen. The reduced mixture was divided into two parts, A and B; part A was worked up under a basic condition and part B was treated with sufficient perchloric acid to remove the methoxide. The capillary vpc analysis (45-m Ucon LB-550-X) showed, besides the 50% recovery of 7-chloronorbornane: in part A, 7-methoxynorbornane, (2-methoxyethyl)cyclopentane (V),[5-7] bp 74° (48 mm), n^{21}D 1.4313, and an unknown compound (VI) in the relative peak areas of 24, 22, and 54, respectively (total yield 97%); in part B, 7-methoxynorbornane, V, and *anti*-7-norbornenol (VII) in the relative areas of 24, 18, and 58, respectively. Therefore, it can be concluded that VI observed in part A



V VI VII

was converted into VII in part B. For isolation of VI, part A was treated with elution chromatography on Merck standardized alumina using a mixed solvent of pentane and ether (99:1). After elution of 7-chloro- and 7-methoxynorbornane and V, a sample of VI, bp 90° (bath temperature) at 110 mm, contaminated with about 15% of V, was obtained.[8] The infrared spectrum of VI in CS_2 showed cyclopropyl CH at 3040

(4) Second-order plots showed upward curvature. The presence of 0.128 *M* sodium perchlorate, instead of sodium methoxide, gave 1.98×10^{-4} sec^{-1}. Therefore, the slightly increased rate in this case over that in the methanolysis without methoxide was ascribed to a salt effect.

(5) Satisfactory analyses were obtained for all compounds described.

(6) Isolated by elution chromatography and identified with the sample independently prepared by methanolysis of β-cyclopentylethyl bromide.[7]

(7) C. R. Noller and R. Adams, *J. Am. Chem. Soc.*, **48**, 2444 (1926).

(8) Standing on the alumina for 2–3 hr induced the isomerization of VI into *anti*-7-methoxynorbornene, bp 80° (25 mm), n^{25}D 1.4630.