Our work in this area is continuing.[10]

(10) We should like to thank Professor A. Horeau for kindly informing us that our method has been independently applied in his laboratory.

(11) National Institutes of Health Predoctoral Fellow, 1964–1965.
(12) National Aeronautics and Space Administration Fellow, 1964–1966.

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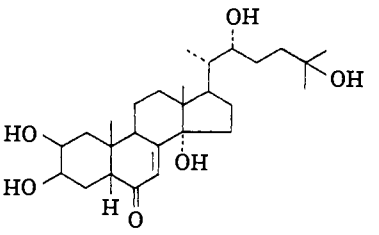
Received January 3, 1966

Steroids. CCXCII.[1] Synthetic Studies on Insect Hormones. II. The Synthesis of Ecdysone

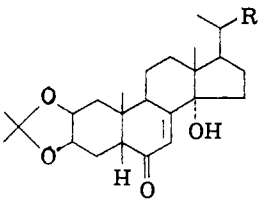
Sir:

We wish to report the completion of the synthesis of the insect moulting hormone ecdysone (Ia),[2] from readily available starting materials, by further transformations of the previously reported[1] methyl 14α-hydroxy-2β,3β-isopropylidenedioxy-6-oxo-22,23-bis-nor-5β-chol-7-enoate (IIa).

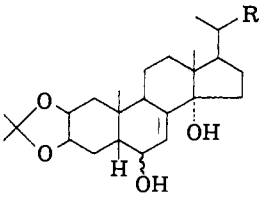
Reduction of IIa with tri-*t*-butoxylithium aluminum hydride afforded a mixture of the corresponding 6α- and 6β-carbinols[3] IIIa which, on alkylation[4] at C-24 with a tetrahydrofuran solution of the lithium salt of 2-methyl-4-sulfoxyphenylbutan-2-ol tetrahydropyranyl ether,[5] furnished 6,14α-dihydroxy-2β,3β-isopropylidenedioxy-25-tetrahydropyranyloxy-23-sulfoxyphenyl-5β-cholest-7-en-22-one [IIIb, $\lambda_{max}^{CHCl_3}$ 5.86 μ; ν^{CDCl_3} 73 (26- and 27-H) and 454 (phenyl H) cps][7] as a mixture of C-6 and side-chain epimers.[3] The crude product was separated from excess alkylating agent by thin layer chromatography on Merck HF silica gel and the phenylsulfinyl group hydrogenolyzed with aluminum amalgam[4] to give IIIc [$\lambda_{max}^{CHCl_3}$ 5.87 μ]. Reduction of this 22-ketone with lithium aluminum hydride to yield the epimeric 22-carbinols IIId followed by manganese dioxide[8] oxidation of the allylic 6-hydroxyl group afforded the epimeric enones IIb [λ_{max}^{MeOH} 240 mμ; $\lambda_{max}^{CHCl_3}$ 5.99 μ; ν^{CDCl_3} 59.5 (19-H), 75 (26- and 27-H), and 350 (7-H) cps]. Hydrolysis of IIb with 0.1 *N* hydrochloric acid in 10% aqueous tetrahydrofuran to remove the acetonide and tetrahydropyranyl ether protecting groups afforded fractions A [15% from IIIa, R_f 0.06; $\lambda_{max}^{THF\ film}$ 6.10 μ; ν 47.5 (18-H), 63.5 (19-H), and 84.5 (26- and 27-H) cps], B [12%, R_f 0.10; ν 44 (18-H), 64 (19-H), and 82 (26- and 27-H) cps], C [6%, R_f 0.14; $\lambda_{max}^{CHCl_3}$ 6.01 μ; ν 48 (18-H), 63.5 (19-H), and 75 (26- and 27-H) cps], D [6%, R_f 0.21; $\lambda_{max}^{CHCl_3}$ 5.86 and 5.98 μ; ν 42.5 (18-H), 62 (19-H), and 129 cps], and E [5%, R_f 0.28; $\lambda_{max}^{THF\ film}$ 6.07 μ; ν 43.5 (18-H), 64.5 (19-H), and 74.5 (26- and 27-H) cps] which were separated on 0.25-mm plates using Merck GF silica gel with 10% methanol in chloroform as the developing system.



Ia
b, 20ξ, 22ξ



IIa, R = COOMe (20α_F)
b, R = CHOHCH$_2$CH$_2$C(CH$_3$)$_2$OTHP



IIIa, R = COOMe (20α_F)
b, R = COCH(SOC$_6$H$_5$)CH$_2$C(CH$_3$)$_2$OTHP
c, R = COCH$_2$CH$_2$C(CH$_3$)$_2$OTHP
d, R = CHOHCH$_2$CH$_2$C(CH$_3$)$_2$OTHP

Fraction D did not show the 26- and 27-H resonances required for the cholestane side chain.[9] Fractions A, C, and E are most probably the three possible C-20 and -22 isomers[10] Ib of ecdysone. Further characterization of these compounds will be the subject of future reports. Fraction B was crystallized from tetrahydrofuran and then water to yield ecdysone [Ia, phase change 165–167°; mp 237–239.5°; [α]D 58° (*c* 0.1, EtOH); λ_{max}^{EtOH} 241 mμ (ϵ 12,300); λ_{max}^{KBr} 2.92 and 6.04 μ; ν_{max} 73 (18-H), 107 (19-H), 124 and 131 (21-H), and 138 (26- and 27-H) cps];[11] identical in all physical properties with those reported by Karlson, *et al.*,[2] for the natural product.[12]

The synthetic material was tested for biological activity by Professor Carroll M. Williams of Harvard University.[13] For this purpose a sample of the

(1) For part I see Steroids. CCXCI: J. B. Siddall, J. P. Marshall, A. Bowers, A. D. Cross, J. A. Edwards, and J. H. Fried, *J. Am. Chem. Soc.*, **88**, 379 (1966).

(2) P. Karlson, H. Hoffmeister, W. Hoppe, and R. Huber, *Ann.*, **662**, 1 (1963); W. Hoppe and R. Huber, *Chem. Ber.*, **98**, 2353 (1965); H. Hoffmeister, C. Rufer, H. H. Keller, H. Schairer, and P. Karlson, *ibid.*, **98**, 2361 (1965); C. Rufer, H. Hoffmeister, H. Schairer, and, M. Traut, *ibid.*, **98**, 2383 (1965); R. Huber and W. Hoppe, *ibid.*, **98**, 2403 (1965).

(3) Separation of the epimers was not deemed necessary since the asymmetry was to be removed in subsequent steps.

(4) E. J. Corey and M. Chaykovsky, *J. Am. Chem. Soc.*, **87**, 1345 (1965).

(5) Prepared from the corresponding carbinol[6] and dihydropyran in the presence of *p*-toluenesulfonic acid.

(6) I. Montanari, R. Danieli, H. Hogeveen, and G. Maccagnani, *Tetrahedron Letters*, 2685 (1964).

(7) Nmr spectra were recorded on a Varian A-60 spectrometer using deuteriopyridine as solvent unless indicated otherwise. We wish to thank Dr. L. Throop, J. Murphy, Dr. T. Toube, and their associates for the determination of physical properties.

(8) Obtained from Beacon Chemical Industries, Inc., Cambridge 40, Mass.

(9) The spectral data suggest a methyl ketone, probably 2β,3β,14α-trihydroxy-24-nor-20ξ-chol-7-ene-6,22-dione.

(10) The formation of all four possible ecdysone side-chain stereoisomers suggests epimerization at C-20 of the methyl ester or the derived 22-ketone. The maintenance of the 19-H resonance at *ca.* 63 cps excludes[1] isomerization at C-5.

(11) This spectrum was recorded on a Varian HA-100 spectrometer using deuteriopyridine as the solvent. We wish to thank Dr. N. Bhacca of Varian Associates for carrying out this determination. The mass spectrum of ecdysone was determined on an Atlas CH-4 spectrometer at a source temperature appreciably below 150° and was in satisfactory agreement with the fragmentation pattern reported by Karlson, *et al.*[2]

(12) The completion of the synthesis of ecdysone from readily available starting materials also completes the formal total synthesis of this natural product.

(13) We are deeply indebted to Professor C. M. Williams for this evaluation.

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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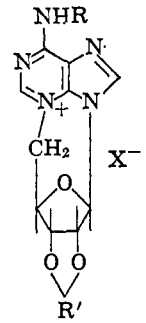
Received January 24, 1966

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$_3$

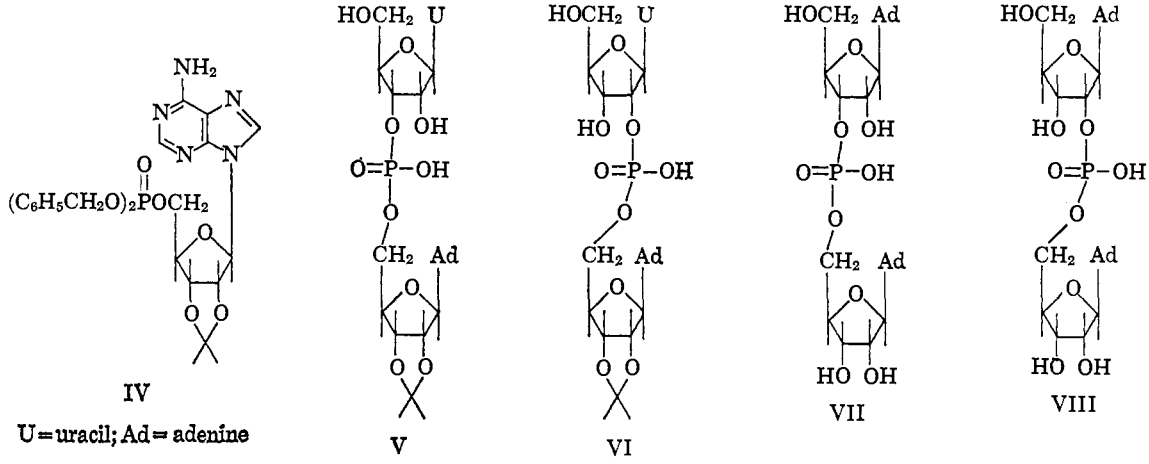
II, R′ = >P(O)–OH, R = H; IIa, R = COCH$_3$

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).