be distinguished in the n.m.r. spectrum at τ 4.3, and the product gave a positive tetranitromethane test.

A solution of 10 mg. of 3α,5-cyclo-4β-methylcholestan-6β-ol in 10 ml. of dioxane containing 1 ml. of 1 *N* sulfuric acid was allowed to stand at room temperature overnight. T.l.c. on the product after work-up indicated that dehydration to 4-methyl-$\Delta^{3,5}$-cholestadiene had taken place. No alcohol was detected.

4β-Methylcholesteryl *p*-toluenesulfonate (50 mg.) in 50 ml. of acetone containing 5 ml. of water and 50 mg. of potassium acetate was kept at reflux for 4 hr. It was worked up in the usual way with the temperature kept as low as possible. The crude product (32 mg.) was dissolved in 50 ml. of ethanol. A quantitative determination of ultraviolet absorption at 239 mμ indicated the presence of 47% 4-methyl-$\Delta^{3,5}$-cholestadiene. The solution was allowed to stand at room temperature for 1 week after which the ultraviolet absorption indicated 78% of the diene. The hydrolysis was repeated with 50-mg. samples of 4β-methylcholesteryl *p*-toluenesulfonate several times and the result was consistent within ±5%. The same result was obtained when the product was allowed to stand on a column of alumina for 6 hr. The percentage of diene (40–50% directly after hydrolysis) rose to 80–85% after elution from the column.

Reduction of Esters of the Windaus Keto Acid by Sodium Borohydride

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The reactions of the keto esters IIb and IIc and the lactone III in 2-propanol with sodium borohydride have been studied intensively. The alkoxyborohydrides formed during the reductions react with 2-propanol yielding hydrogen. A boron-containing material has been isolated. These results, which are due to side reactions, and the mechanism of reduction of ester groups by borohydride are discussed.

Some years ago Schenker[3] suggested that the reduction of esters and lactones by sodium borohydride requires the presence of other groups such as hydroxyl. This view is in accord with a recent result[4] that a fully esterified sugar lactone gave only very poor yields of hemiacetal on treatment with borohydride.[5] In 1961, Atwater[7] obtained complex mixtures of lactones, hemiacetals, and in some cases diols from the borohydride reduction of δ-keto esters derived from the cleavage of ring A or ring B of steroids. He concluded that the formation of the lactones was a prerequisite for further reduction (*cf.* ref. 8). In previous work,[9] we reduced a γ-keto ester with sodium borohydride to a mixture which included two epimeric diols. As the formation of a lactone intermediate *en route* to the predominant diol was unlikely for steric reasons, we proposed that for this compound, at least, the reduction of the ester group might proceed by way of an intramolecular transfer of a hydride ion (I, see Chart I). This mechanism involves an intramolecular reduction with an alkoxyborohydride, which should be a more powerful reducing agent than borohydride itself.[10] The results obtained by Barnet and Kent[11] support our suggestion. They found that under comparable conditions the yield of diol from borohydride reduction increases as one goes from γ- to β- to α-keto esters; an intramolecular transfer of hydride (*cf.* I) would involve five- and six-membered rings with the α- and β-keto compounds, respectively.

While working on another problem,[12] we observed that reduction of the keto ester IIb[13] with sodium borohydride gave a mixture of products including the lactone III,[13,14] the acetal IVb,[15] and the diol V.[14] These products were obtained as mixtures of stereoisomers at the 5-position. There appeared to be two possible paths for the reductions: II → VI → III → IVa → VII → V and II → VI → IX (*via* VIII) → VII → V. We decided to study the reaction by measuring the rate of disappearance of ketone in the reduction of IIb and IIc and by determining the rate of hydride consumption and carrying out product studies on the reductions of IIb, IIc, and III. From these studies we hoped to deduce whether III was an intermediate in the formation of IVb and V. The reactions proved to be more complicated than we had expected, and we could not attain our original objectives. However, the complications are of some intrinsic interest and so we report our results here.

Results

The reductions of the methyl ester IIb, the isopropyl ester IIc, and the lactone III were carried out in solutions of 2-propanol; 2-propanol, unlike ethanol or methanol, does not react with sodium borohydride.[16,17] The courses of the reductions were followed by observing the disappearance of ketone (from the changes in the ultraviolet maxima at 290 mμ) and the consumption of hydride (by titration). The reductions of the keto esters were carried out using 1 mole and 0.25 mole equiv. of sodium borohydride. The reduction of the lactone, which could be followed by titration only, was studied using 1 mole equiv. of borohydride.

(1) American Chemical Society Petroleum Research Fund Fellow, 1960–1963.

(2) The initial phases of this work were carried out at the Department of Chemistry at the University of South Carolina.

(3) E. Schenker, *Angew. Chem.*, **73**, 81 (1961).

(4) P. Kohn, R. H. Samaritano, and L. M. Lerner, *J. Am. Chem. Soc.*, **86**, 1457 (1964).

(5) Brown and Rapoport[6] have observed that esters are reduced to primary alcohols using a large excess of sodium borohydride in methanol. However, under these conditions the effective reducing agent is probably trimethoxyborohydride rather than borohydride itself.

(6) M. S. Brown and H. Rapoport, *J. Org. Chem.*, **28**, 3261 (1963).

(7) N. W. Atwater, *J. Am. Chem. Soc.*, **83**, 3071 (1961).

(8) H. O. House, H. Babad, R. B. Toothill, and A. W. Noltes, *J. Org. Chem.*, **27**, 4141 (1962).

(9) D. M. S. Wheeler and M. M. Wheeler, *ibid.*, **27**, 3796 (1962).

(10) H. C. Brown, E. J. Mead, and C. J. Shoaf, *J. Am. Chem. Soc.*, **78**, 3616 (1956).

(11) J. E. G. Barnet and P. W. Kent, *J. Chem. Soc.*, 2743 (1963).

(12) E. C. Pesterfield, Ph.D. Thesis, University of South Carolina, 1965.

(13) R. B. Turner, *J. Am. Chem. Soc.*, **72**, 579 (1950).

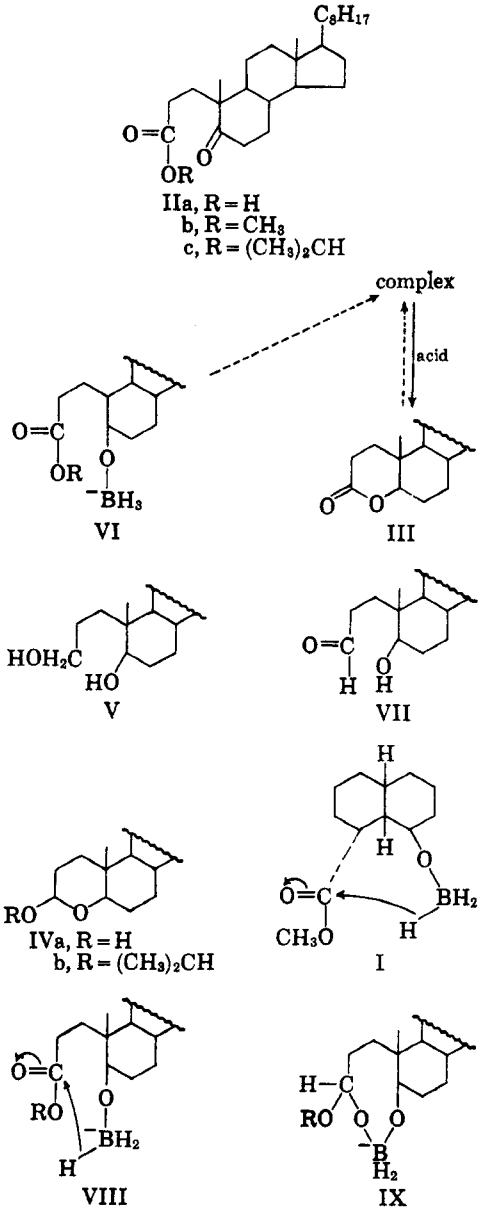
(14) J. T. Edward and P. F. Morand, *Can. J. Chem.*, **38**, 1325 (1960).

(15) J. T. Edward and I. Puskas, *ibid.*, **40**, 711 (1962).

(16) H. C. Brown, E. J. Mead, and B. C. Subba Rao, *J. Am. Chem. Soc.*, **77**, 6209 (1955).

(17) H. C. Brown and K. Ichikawa, *ibid.*, **83**, 4372 (1961).

CHART I



The initial second-order rate constants for the various reactions (see Experimental for figures) were deduced by an extrapolation method.[12] While the figures for the reductions of the methyl ester IIb and the lactone III are reasonably consistent, those for the isopropyl ester IIc varied somewhat. The variations are probably a result of the difficulty of satisfactorily estimating borohydride in the presence of the products of the reactions. The rate constants for the reductions of the keto esters IIb and IIc at 23.5° are almost the same as that for the reduction of acetone at 25°.[18] The rate constants for the reduction of the lactones are one-third (or less) of those of the keto esters.

The results of our product studies are summarized in Table I. The details and limitations of this work are discussed in the Experimental. As indicated earlier the main products of our reactions were the lactone III, the acetal IVb, and the diol V. We made no attempt to separate stereoisomers, though we characterized the major stereoisomers, the 5α-H epimers

(18) H. C. Brown, O. H. Wheeler, and K. Ichikawa, *Tetrahedron*, **1**, 214 (1957).

TABLE I

PRODUCT STUDIES

Run	Time, min.	% keto ester—Ultraviolet	% keto ester—Found	% lactone found	% acetal found	% diol found	% column recovery
Reduction of Methyl Ester (0.0513 *M*) with $NaBH_4$ (0.0620 *M* in H^-)							
1	57	80	70	10			80
2	64	76	61	26			87
1	248	51	48	30	3		81
2	251	50	50	27	7		84
1	720	31	29	48	16		93
2	760	30	31	45	17		94
1	1410	26	26	46	16		89
2	1450	26	18	36	18		72
Reduction of Methyl Ester (0.0577 *M*) with $NaBH_4$ (0.2309 *M* in H^-)							
3	30	65	50	14	7		72
4	30	68	55	17	6		78
3	80	35	16	47	21		84
4	80		21	46	22		89
4	353			40	43	8	92
3	402			40	38	18	96
4	1128			37	13	44	94
3	1177			36	8	40	84
Reduction of Isopropyl Ester (0.0508 *M*) with $NaBH_4$ (0.0508 *M* in H^-)							
5	59	79	81	20			101
6	59	79	78	13			91
5	210	57	67	29			96
6	254	54	57	36	7		100
5	380	48	34	55	5		95
6	414	44	26	46			72
5	1370		24	60	12		96
6	1404		26	40	11		77
Reduction of Isopropyl Ester (0.0522 *M*) with $NaBH_4$ (0.2088 *M* in H^-)							
7	30	68	51	45			96
8	30	69	40	42	7		89
7	68	45	40	47	11		98
8	73	49	30	55	12		96
7	300		10	44	31	9	95
8	353		7	53	27	16	103
7	1080			34	9	49	92
8	1143		2	27	6	33	67
Reduction of Lactone (0.0522 *M*) with $NaBH_4$ (0.2452 *M* in H^-)							
9	60			78	16	3	96
9	293			70	29	5	104
9	562			27		71	98
9	1233			22		71	93
Reduction of Lactone (0.0522 *M*) with $NaBH_4$ (0.210 *M* in H^-)							
10	53			90	9		99
10	253			74[a]	12	14	100
10	403			38	20	41	99
10	1933			32		68	100

[a] Contains hemiacetal.

of these compounds. The acetal is mainly the 3α-isopropoxy compound; this is probably not significant as the 3α isomer predominates at equilibrium.[15] We believe that the acetal IVb is not present as such in the reaction mixture but is formed from the hemiacetal IVa during the work-up of the products.[19]

A study of the material balances of all the reactions indicated that more hydride was always consumed (as determined by titration) than could be accounted for

(19) Although we did not isolate the hemiacetal IVa during the product studies, examination of infrared spectra of the fractions containing lactone from the chromatograms suggested that traces of it were usually present.

TABLE II

HYDROGEN EVOLUTION EXPERIMENTS[a]

Run	Time, min.	% lactone found	% acetal found	% diol found	% column recovery	H^- (mequiv.) required for products	H_2 (ml. at STP) evolved	H^- (mequiv.) required for H_2	Total H^- (mequiv.) required	H^- (mequiv.) consumed
Methyl 1	1360	40	12	47	99	2.46	8.7	0.39	2.85	2.66
Methyl 2	1360	37	11	43	91	2.26	8.7	0.39	2.65	2.47
Isopropyl 1	1360	32	10	48	90	2.38	8.2	0.37	2.75	2.76
Isopropyl 2	1360	32	19	38	89	2.23	8.7	0.39	2.62	2.36
Lactone 1	1360	23		67	90	1.63	6.4	0.29	1.92	1.96
Lactone 2	1360	18		72	90	1.75	6.4	0.29	2.04	1.76

[a] Esters were 0.0526 M, $NaBH_4$ was 0.2096 M in H^-, and lactone was 0.0535 M.

by the reaction products. We accounted for the "missing" hydride by observing that hydrogen was evolved during the reactions. We then carried out a series of reactions in which the hydrogen evolved over 24 hr. was collected and the crude reduction products were chromatographed. The results of these experiments are given in Table II. The figures show that about 15% of the hydride consumed was accounted for by the hydrogen evolved.

In each of the reactions involving 1 mole equiv. of borohydride (runs 3, 4, 7, 8, and the lactone reductions) a precipitate formed which was easily seen after the reaction had proceeded for 90 min.[20] The precipitates from the reductions of IIb, IIc, and III had very similar infrared spectra and were essentially the same material. The spectra did not show the presence of a lactone or a ketone group but suggested that the material contained a carboxylate anion and boron–oxygen bonds. Titration indicated less than 0.1% hydride. Acidifying the precipitates from the reduction of the esters IIb and IIc gave the lactone III (about 50% yield). The analyses of these precipitates did not agree with each other although both indicated about 5% boron. While we are unable to suggest a definite structure for the precipitate, it is probably a boron derivative of the initial reduction product VI in which all hydride hydrogens have been replaced by isopropoxy groups; it may be a mixture of compounds. Presumably the material tied up in the precipitate did not undergo further reduction and was converted to the lactone when the reaction mixture was acidified. In every reaction that involved a mole equivalent of borohydride (see Tables I and II) the yield of acetal reached a maximum (around 300 min.) and then decreased. This was most marked in the reductions of the lactones (in which the hydride is in greatest excess). Further, the recovery of lactone seems to drop to a limiting value of about 20%. The amounts of precipitates we isolated suggest that the limiting yield of lactone should be about 10% for the reductions of the keto esters and perhaps a little less for the reductions of the lactone.

Discussion

The distributions of products (Tables I and II) obtained from the reductions of IIb, IIc, and III are rather similar and suggest a common pathway for the three reactions. However, the interpretation of our results is complicated by two factors. Firstly about 15% of the hydride consumed is accounted for by the production of hydrogen. Brown and his coworkers[16,17] observed (and we confirmed) that borohydride does not react with 2-propanol. The evolution of hydrogen is best accounted for by a reaction of 2-propanol with the sodium alkoxyborohydride species formed during the reduction of the ketone group. It is well known[10,16,18] that alkoxyborohydrides are more reactive than borohydride itself. Further, Davis and Gottbrath[21] obtained parallel results in studies in methanol. The reaction of 2-propanol with the alkoxyborohydrides obviously complicated the course of our reduction by adding a number of isopropoxyalkoxyborohydride species to the system and by taking up some of the hydride in side reactions.

The second complication was the formation of the precipitate, which removed a portion of the reactants from the reaction system after the first reduction stage. Also the lactone isolated from the reaction must come from both precipitated and nonprecipitated material. As the precipitate was recovered from the reduction of the lactone and it does not contain a lactone ring, at least a portion of the lactone must have undergone a ring opening. Thus, if the lactone is an intermediate in the reduction, it must be in equilibrium with VI or some other precursor of the precipitate. If the lactone is not an intermediate, then in the reduction of III all the material is converted to VI; a portion of VI is then converted to the precipitated material, and the bulk undergoes further reduction. While our evidence shows clearly the existence of competing reactions in the reduction, it does not enable us to decide between the mechanistic possibilities. However, on the basis of other work[3,4,9,11] we consider that the lactone III is not an intermediate in the formation of IVb and V; our present results are consistent with this view.

Experimental[22]

A-Nor-3,5-secocholestan-5-on-3-oic Acid Isopropyl Ester (IIc).—A solution of A-nor-3,5-secocholestan-5-on-3-oic acid[24] (IIa,

(20) There is fair agreement in runs 1, 2, 5, and 6 (involving the 0.25 mole equiv. of sodium borohydride) between the amount of keto ester recovered from the reaction and that deduced from the ultraviolet spectrum. In runs 3, 4, 7, and 8 the recovery of keto ester was always much less than would be expected from the ultraviolet spectrum. The discrepancy is due, presumably, to the precipitate, which increased the optical density of the solution.

(21) R. E. Davis and J. A. Gottbrath, *J. Am. Chem. Soc.*, **84**, 895 (1962).

(22) Ultraviolet spectra of 2-propanol solutions were measured on a Cary spectrophotometer, Model 11. Infrared spectra of carbon tetrachloride solutions (unless otherwise stated) were determined on Perkin-Elmer instruments, Models 21, 137, and 237. 2-Propanol was Fisher Certified reagent (water 0.03%). Florisil was 60–100 mesh. Sodium borohydride for quantitative work was analytical grade from Metal Hydrides, Inc. It was supplied as 99+%; on assay[23] we found it to be 98.6% before and 98.5% after our runs. Melting points are not corrected.

(23) D. A. Lyttle, E. H. Jensen, and W. A. Struck, *Anal. Chem.*, **24**, 1843 (1952).

(24) J. T. Edward, D. Holder, W. H. Lunn, and I. Puskas, *Can. J. Chem.*, **39**, 599 (1961).

10 g.) and *p*-toluenesulfonic acid (100 mg.) in 2-propanol was refluxed for 40 hr. The solution was concentrated to one-half volume, ether was added, and the ethereal solution was washed with water, aqueous potassium carbonate, and water and was dried. Removal of the solvent gave the isopropyl ester IIc which crystallized from aqueous 2-propanol as needles: 6.6 g.; m.p. 56–56.5°; λ_{max} 293 mμ (ε 24.8); ν 2940, 2865, 1732, 1708, 1477, 1434 (sh), 1382, 1299, 1187, 1152, 1118, 1010, and 958 cm.$^{-1}$.

Anal. Calcd. for $C_{29}H_{50}O_3$: C, 77.97; H, 11.28; O, 10.75. Found: C, 78.28; H, 11.05; O, 10.99.

A-Nor-3,5-secocholestan-5-on-3-oic Acid Methyl Ester (IIb).—The methyl ester IIb,[13] which was obtained from the acid IIa (10 g.) by treatment with diazomethane, was passed through an alumina column and eluted in acetone. It was obtained as an oil: 8.2 g.; b.p. 100–110° (10^{-5} mm.) with some decomposition; λ_{max} 292 mμ (ε 27.9); ν 2950, 2870, 1740, 1707, 1475, 1454, 1446, 1384, 1304, 1199, 1177, 1033, and 957 cm.$^{-1}$.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08; O, 11.47. Found: C, 77.56; H, 11.12; O, 11.52.

Reduction Products. A. 4-Oxa-5α-cholestan-3-one (III).—The lactone III, prepared by the method of Edward and Morand[14] had m.p. 113–115° (lit.[14] m.p. 114–115°); ν 2950, 2870, 1745, 1475, 1455, 1390, 1380, 1372, 1347, 1195, 1136, 1083, and 1054 cm.$^{-1}$ (corresponds with the spectrum reported by Edward[14]).

Anal. Calcd. for $C_{26}H_{44}O_2$: C, 80.35; H, 11.41; O, 8.23. Found: C, 80.57; H, 11.30; O, 8.41.

B.—The identities of the other reduction products, A-nor-3,5-secocholestan-3,5β-diol (V) and 3-isopropoxy-4-oxacholestane (IVb), were confirmed by comparison with authentic samples[14,15] (by infrared spectrum and mixture melting point for V and infrared spectrum for IVb).

Reaction Runs.—The reductions of the esters IIb and IIc in 2-propanol at 23.5° were studied using 1 mole and 0.25 mole equiv. of borohydride; the lactone was studied under similar conditions using 1 mole equiv. of borohydride. A saturated solution of sodium borohydride in 2-propanol was filtered, a little 2-propanol was added, and the concentration of hydride was determined.[23] The concentration of borohydride did not change on standing for 24 hr. In the presence of keto ester the titrations became a little erratic; this causes an uncertainty of about 5% in our estimations of borohydride during the reactions. From an over-all inspection of results the hydride consumption as determined by titration was probably low (*cf.* Table II). The concentration of keto ester (IIb or IIc) during the reaction was deduced from the ultraviolet absorption peak at 290 mμ.

The solutions of the ester and sodium borohydride were mixed at $t = 0$ and a portion of the mixture was immediately transferred to a thermostated cell (23.5°) in the ultraviolet spectrophotometer. At appropriate times samples (1.95 ml.) were withdrawn from the main solution and the borohydride concentration was estimated.[22] For each run nine such estimations were done; six of them were within the first hour. The initial rate constants for these reactions were deduced by extrapolation methods described elsewhere.[12] The initial second-order rate constants[12] (ester and hydride concentrations) varied from 0.9×10^{-3} to 1.4×10^{-3} l. mole^{-1} sec.$^{-1}$ for the reduction of the methyl ester IIb (four runs), and from 0.9×10^{-3} to 5.6×10^{-3} (four runs) for the reduction of the isopropyl ester IIc. The reduction of the lactone III was studied in a similar way by following the consumption of hydride. The second-order constants (lactone and hydride) for the reaction which were also deduced by an extrapolation method[12] varied from 3.1×10^{-4} to 3.3×10^{-4} l. mole^{-1} sec.$^{-1}$ (two runs).

Product Studies for Reductions of IIb, IIc, and III.—Samples (usually 3.5 ml.) withdrawn from the reaction mixture at appropriate times were immediately acidified and ether was added. The ethereal solution was washed with water (three times and was dried (Na_2SO_4). Removal of the solvent gave the crude product. Making no allowance for changes in molecular weights the yields averaged 86% for reactions of the methyl ester IIb, 70% for the isopropyl ester IIc, and 92% for the lactone III. Allowing for the changes in molecular weight the yields would become (approximately) 90, 80, and 92%, respectively. The crude products were chromatographed on Florisil (usually 15 g.) packed in a column 10 mm. in diameter. The following amounts of solvents were passed through and 15-ml. fractions taken: benzene (60 ml.) and benzene–ethyl acetate (50:1, 60 ml.; 25:1, 60 ml.; 9:1, 60 ml.; and 1:1, 60 ml.). The isopropyl acetal IVb was eluted in benzene; the esters IIb and IIc in 50:1 and 25:1 benzene–ethyl acetate; the lactone III in 9:1 ethyl acetate–benzene; and the diol V in benzene–ethyl acetate (1:1). The conditions for separation on the columns were worked out by careful monitoring of the fractions by infrared spectra. During later runs fractions were checked from time to time by their infrared spectra. The recovery of material from the columns averaged 90%. The results are summarized in Table I.

In our columns the separations of the acetal and diol from the other fractions were good. The separation of the lactone from the keto ester was never quite so satisfactory and the cuts were made at the minimum points in the chromatography curve. In addition the infrared spectra indicated that traces of hemiacetal IVa[25] were usually present in the lactone fractions. In spite of these difficulties our figures show a general consistency between similar runs,[27] we believe that the results give at least a good qualitative idea of how the product distributions vary with time during the reductions.

Hydrogen Evolution Experiments.—Experiments were run in duplicate using the keto esters IIb and IIc and the lactone III. Solutions of the compounds (500 mg. of methyl ester IIb; 533 mg. of isopropyl ester IIc; 474 mg. of lactone III) in 2-propanol (14.5 ml.) were treated with solutions of sodium borohydride (1.19 mmoles) in 2-propanol (8.3 ml.). Each solution at 23.5° was connected to a gas buret (which was left under a slightly negative pressure). After 1360 min. the buret was under positive pressure and the volume of gas evolved was measured; a sample (1.95 ml.) of the solution was withdrawn and the concentration of hydride was estimated by titration.[23] The gas evolved was colorless, odorless, and burned on lighting. The reaction solution was acidified and worked up, and samples (about 40–80 mg.) of the crude products were chromatographed on Florisil. The results are summarized in Table II.

Isolation of Boron Complex Formed during Reduction. A.—A solution of the methyl ester IIb (500 mg.) in 2-propanol (6.9 ml.) was treated with sodium borohydride (1.19 mmoles) in 2-propanol (15.9 ml.) and was kept at 23.5°. The separation of a precipitate was observed after 90 min. After 26 hr. the precipitate (76 mg.) was collected and dried; the filtrate was acidified and worked up in the usual manner to yield crude product (358 mg.). The precipitate did not melt below 280°; its infrared spectrum (ν^{KBr} 3400, 2940, 2860, 1565, 1410, 1030, and 875 cm.$^{-1}$) was almost identical with the spectra of the precipitates obtained from the reductions of the isopropyl ester IIc and the lactone III. There was a slight absorption at 2300 cm.$^{-1}$ which was also observed in C below.

Anal. Found: C, 51.87; H, 8.16; B, 5.42.

A portion of the precipitate (54 mg.) was treated with acid and the acidified solution was extracted with ether. The ethereal solution was washed with water and dried (Na_2SO_4). Removal of the solvent gave a solid (39 mg.); a portion of this (26 mg.) was chromatographed on Florisil (10 g.); elution with benzene–ethyl acetate (25:1 and 9:1) gave solid material (19 mg.) identified as the lactone III by its infrared spectrum.

Three portions (80, 55, and 79 mg.) of the crude product from the filtrate were chromatographed on Florisil in the usual way. None of the columns was completely satisfactory as the recovery of products was 74, 74, and 78%. The average per cent composition was 14% acetal, 36% lactone, and 25% diol.

B.—A similar experiment was carried out using isopropyl ester (500 mg.), sodium borohydride (1.12 mmoles), and 2-propanol (21.5 ml.). After 48 hr. at 28° the precipitate (107 mg.) was collected. Its infrared spectrum was almost identical with that of the precipitate from A.

Anal. Found: C, 44.83; H, 7.34; B, 5.56.

A sample (35 mg.) of the precipitate was acidified and worked up as described above, and the lactone III (19 mg.), identified by infrared spectrum, was obtained. Work-up of the mother liquors from the reduction gave crude product (337 mg.).

(25) We obtained authentic hemiacetal as a by-product in a preparation of the lactone III; our material had the same melting point and infrared spectrum as had been reported by Edward, Morand, and Puskas.[26]

(26) J. T. Edward, P. F. Morand, and I. Puskas, *Can. J. Chem.*, **39**, 2069 (1961).

(27) Apart from the results reported here (which were obtained using sodium borohydride of about 99% purity), we also carried out a considerable amount of work with borohydride of lesser purity. The results[12] of this work, which involved about 60 chromatograms, fully support our present figures.

C.—A similar reaction was carried out with the lactone III (423 mg.). After 24 hr. the precipitate (40 mg.) was collected; it had almost the same infrared spectrum as the precipitates from A and B; as with the material from A there was a slight absorption at 2300 cm.$^{-1}$ indicating the presence of a trace of B–H. Samples of the precipitate were titrated[23] for hydride and the hydride content was estimated as 0.06 and 0.09%.

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Phosphonic Acid Analogs of Nucleoside Phosphates. III. The Synthesis of Adenosine-5′-methylenediphosphonate, a Phosphonic Acid Analog of Adenosine-5′-diphosphate[1,2]

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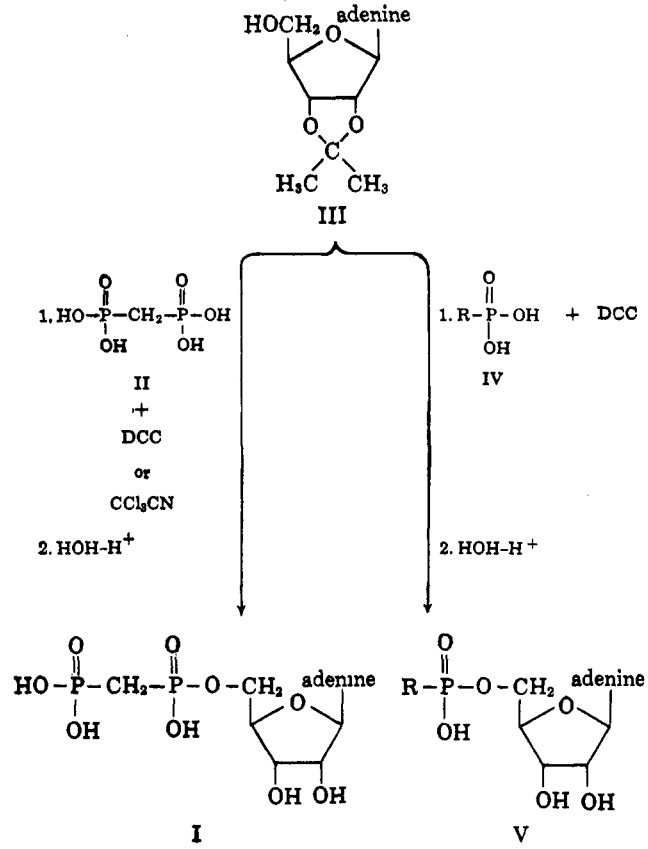
The synthesis of adenosine-5′-methylenediphosphonate (I) has been accomplished *via* the reaction of 2′,3′-isopropylideneadenosine with methylenediphosphonic acid as mediated by trichloroacetonitrile and by dicyclohexylcarbodiimide. Adensoine-5′-methylphosphonate (V, R = CH_3) and -5′-ethylphosphonate (V, R = CH_2CH_3) were prepared in an analogous fashion with use of dicyclohexylcarbodiimide.

The development of methods for the synthesis of nucleoside esters of phosphonic acids has been undertaken in these laboratories as part of a program on the preparation and biological properties of phosphonic acid analogs of nucleoside phosphates.[3]

The work relating to the synthesis of adenosine-5′-methylenediphosphonate (I) is presented in the present paper. This compound, an analog of ADP in which the pyrophosphate oxygen has been replaced with a methylene group, is an adenosine ester of methylenediphosphonic acid (II). Accordingly, methods used for the synthesis of phosphate esters of nucleosides were investigated for its preparation. These include the esterification of methylenediphosphonic acid with 2′,3′-isopropylideneadenosine (III) as mediated by dicyclohexylcarbodiimide (DCC) and by trichloroacetonitrile followed by removal of the protecting isopropylidene grouping by acid hydrolysis.[4]

The dicyclohexylcarbodiimide esterification of two alkylmonophosphonic acids, methyl- and ethylphosphonic acid (IV, R = CH_3 and R = CH_2CH_3), was studied as a preliminary to the work with the more complicated methylenediphosphonic acid.[5] Both of these acids smoothly underwent esterification in anhydrous pyridine to give, after removal of the isopropylidene group, adenosine-5′-methylphosphonate (V, R = CH_3) and adenosine-5′-ethylphosphonate (V, R = CH_2CH_3) as crystalline solids in yields of 86 and 54%, respectively.[6] These esters were characterized by elemental analysis and by equivalent weight estimation from ultraviolet absorbancy measurements.



Analogously the synthesis of adenosine-5′-methylenediphosphonate (I) was accomplished by the re-

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(2) The following abbreviations are used: ATP, adenosine-5′-triphosphate; ADP, adenosine-5′-diphosphate; AMP, adenosine-5′-phosphate; and DCC, dicyclohexylcarbodiimide.

(3) Paper I: T. C. Myers, K. Nakamura, and J. W. Flesher, *J. Am. Chem. Soc.*, **85**, 3292 (1963); paper II: T. C. Myers and L. N. Simon, *J. Org. Chem.*, **30**, 443 (1965).

(4) For procedures analogous to those used in the dicyclohexylcarbodiimide esterifications, see (a) D. B. Straus and E. Goldwasser, *Biochim. Biophys. Acta*, **47**, 186 (1961); (b) M. Smith and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 1141 (1958); (c) H. G. Khorana, "Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest," John Wiley and Sons, Inc., New York, N. Y., 1961, Chapters 2, 5, and 6; to those used in the trichloroacetonitrile esterification, see (d) F. Cramer, K. H. Scheit, and H. J. Baldauf, *Chem. Ber.*, **95**, 1657 (1962); (e) F. Cramer and H. J. Weimann, and G. Weimann, *ibid.*, **94**, 996 (1961).

(5) Dicyclohexylcarbodiimide-mediated esterification of high molecular weight alkylmonophosphonic acids has been recently reported by J. A. Maynard and J. M. Swan [*Australian J. Chem.*, **16**, 609 (1963)].

(6) The preparation of adenosine-5′-ethylphosphonate as an amorphous solid has been previously reported *via* reaction of isopropylideneadenosine with benzyl ethylphosphonochloridate followed by removal of the protecting groups: N. Anand and A. R. Todd, *J. Chem. Soc.*, 1867 (1951).