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Synthesis of 9-β-D-Arabinofuranosylguanine*

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Received July 3, 1963

The synthesis of 9-β-D-arabinofuranosylguanine is described. The fusion reaction of 2,6-dichloropurine and xylofuranose tetraacetate gave, after ammonolysis and acetonide formation, crystalline 6-amino-2-chloro-9-(3′,5′-*O*-isopropylidene-β-D-xylofuranosyl)-9H-purine. Using the conventional xyloside-arabinoside conversion scheme, 6-amino-2-chloro-9-(β-D-arabinofuranosyl)-9H-purine was prepared from 6-amino-2-chloro-9-(3′,5′-*O*-isopropylidene-β-D-xylofuranosyl)-9H-purine. Deamination of 6-amino-2-chloro-9-(β-D-arabinofuranosyl)-9H-purine gave crystalline 2-chloro-6-hydroxy-9-(β-D-arabinofuranosyl)-9H-purine which was ammonolized to give the title compound.

The β-D-arabinosyl derivatives of uracil, thymine, and cytosine, naturally occurring pyrimidine nucleic acid bases, have been synthesized and have shown interesting biological activity. Chu and Fischer (1962) observed that arabinosylcytosine inhibited the conversion of cytidylic acid to 2′-deoxycytidylic acid. Evans *et al.* (1961) reported that arabinosylcytosine was a potent inhibitor of the growth of tumor cells in tissue culture and also caused striking regression of well-established tumors in mice. Pizer and Cohen (1960) observed that arabinosyluracil was not cleaved by enzymes that rupture the base-sugar bond of uridine, although it was phosphorylated to the nucleotide by the enzymes that phosphorylated deoxyuridine.

More recently, arabinofuranosyladenine has been synthesized (Lee *et al.*, 1960; Reist *et al.*, 1962) and its biological activity has been investigated. Hubert-Habert and Cohen (1962) demonstrated that arabinofuranosyladenine inhibits DNA synthesis in a purine-requiring strain of *E. coli*. In addition, arabinofuranosyladenine appeared to have affected protein synthesis as evidenced by the apparent absence of growth. Evidence was presented (Hubert-Habert and Cohen, 1962) which suggested that arabinofuranosyladenine was located primarily in the terminal nucleotide of s-RNA, a position which would be expected to have a profound influence on protein synthesis. Brink and LaPage (1963) reported that arabinofuranosyladenine inhibited the growth of a number of animal tumors and drastically lowered the incorporation of adenine-C^{14}, guanine-C^{14}, orotic acid-C^{14} and glycine-C^{14} into nucleic acid, especially DNA.

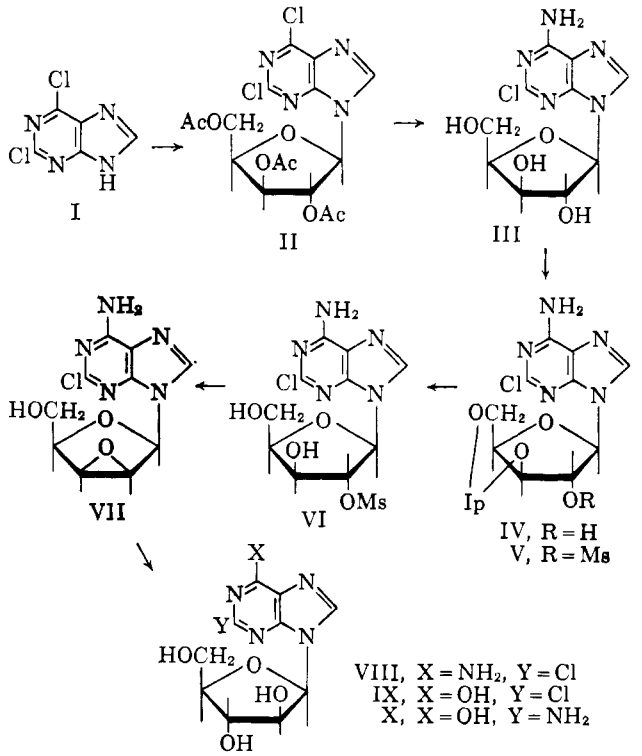
With these data in mind, it was of interest to synthesize the β-D-arabinofuranoyl derivatives of guanine and thus to complete the series of the β-D-arabinosyl derivatives of the common nucleic acid bases.

* This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and are not necessarily those of the Cancer Chemotherapy National Service Center. The authors are indebted to Dr. Peter Lim and staff for the paper chromatograms, optical rotations, ultraviolet spectra, and interpretation of the infrared spectra; and to Mr. O. P. Crews and his staff for the large-scale preparation of intermediates.

Guanine or a blocked derivative has not been successfully condensed directly with a sugar to give a 9-substituted nucleoside. Similarly, an acylated arabinofuranose derivative cannot be coupled directly with a purine or pyrimidine to give a β nucleoside, since the nucleoside condensation inevitably gives, as the predominant product, the anomer in which the steric relationship between the C_2′-hydroxyl and the C_1′-base is *trans* (Baker, 1957). Hence, D-arabinose in such a nucleoside condensation gives the α anomer rather than the desired β anomer (Bristow and Lythgoe, 1949). Thus indirect methods must be used, both to introduce the guanine moiety and to obtain the β linkage of 9-β-D-arabinofuranosylguanine (X).

The synthetic methods for preparing a β-arabinosylnucleoside were developed for the synthesis of arabinosyladenine (Lee *et al.*, 1960; Reist *et al.*, 1962), and were employed in the present work. A number of syntheses of guanosine have been reported (Davoll *et al.*, 1948; Davoll and Lowy, 1951; Davoll, 1958) and a modification of one of these (Davoll *et al.*, 1948) which utilized 2,6-dichloropurine (I) as the guanine precursor was used in the work under discussion. Compound I was coupled with xylofuranose tetraacetate using the elegant nucleoside fusion technique of Shimadate *et al.* (1961) to give the crude β-xylosyl derivative (II). Treatment of compound II with methanolic ammonia simultaneously deblocked the sugar portion and ammonolized the 6-chlorine to give the 2-chloro-6-aminonucleoside (III). This ammonolysis is based on the difference in reaction to nucleophiles between the 2-chlorines of 2,6-dichloropurine as noted by Montgomery and Holum (1957) and Schaeffer and Thomas (1958). Without isolation, compound III was converted with acetone and ethanesulfonic acid to crystalline 6-amino-2-chloro-9-(3′,5′-*O*-isopropylidine-β-D-xylofuranosyl)-9H-purine (IV), isolated in 35% over-all yield from compound I. Hydrogenation of compound IV over 5% palladium-on-charcoal gave crystalline 9-(3′,5′-*O*-isopropylidene-β-D-xylofuranosyl)-adenine which was identical in all respects with the authentic material (Reist *et al.*, 1962). This demonstrated unequivocally that the fusion reaction proceeded in the expected fashion to give a 9-substituted β-nucleoside.

Mesylation of compound IV gave the 2′-*O*-mesylate (V) in 95% yield. Treatment of compound V with



aqueous acetic acid removed the isopropylidene group of compound V and the resulting compound VI was treated with methanolic sodium methoxide to give the epoxide (VII). Reaction of compound VII with sodium acetate in aqueous *N,N*-dimethylformamide afforded 6-amino-9-β-D-arabinofuranosyl-2-chloro-9H-purine (VIII) in 57% yield from compound V. The sugar moiety of nucleoside (VIII) was assigned an arabinose configuration on the basis of the work described previously (Reist *et al.*, 1962) in preparing the β-arabinoside of adenine. That this assignment was correct was proven by hydrogenation of compound VIII to give a product which was chromatographically identical with 9-β-D-arabinofuranosyladenine and significantly different from 9-β-xylofuranosyladenine which is the other alternative after hydrogenation of the product from reaction of sodium acetate with compound VII.

Deamination of compound VIII with nitrous acid gave the crystalline 9-β-D-arabinofuranosyl-2-chloro-6-hydroxy-9H-purine (IX). Treatment of compound IX with methanolic ammonia gave the desired 9-β-D-arabinofuranosylguanine (X) in 16% over-all yield from compound VIII. The similarity of ultraviolet spectra between compound X and guanosine (Beaven *et al.*, 1955) confirms that compound X is indeed a 9-substituted guanine.

Experimental

Melting points were taken on a Fischer-Johns apparatus. Paper chromatograms were run by the descending technique on Whatman No. 1 paper in the following solvent systems: A, water-saturated *n*-butyl alcohol; B, 5% aqueous disodium hydrogen phosphate; C, *n*-butyl alcohol–acetic acid–water (4:1:5). Adenine was used as a standard and spot locations were expressed as R_{Ad} units with adenine at 1.00. The nucleoside spots were located by visual examination under ultraviolet light.

*1,2,3,5-Tetra-O-acetyl-*D*-xylofuranose.*—To a solution of 15.3 g of 3,5-di-*O*-acetyl-1,2-*O*-isopropylidene-D-xylofuranose (Levene and Raymond, 1933) in 200 ml of glacial acetic acid and 23 ml of acetic anhydride was added 14 ml of concentrated sulfuric acid dropwise with stirring and sufficient cooling to maintain a temperature of about 20°. After the addition was complete, the reaction was left at room temperature for 18 hours, and was then poured into about 500 ml of ice water slowly and with stirring. The aqueous layer was extracted with two 300-ml portions of chloroform. The chloroform extract was washed with 400 ml of aqueous sodium bicarbonate and 200 ml of water, dried over magnesium sulfate, and evaporated to dryness *in vacuo* to give 15.5 g (89%) of product as a pale yellow liquid; λ_{max}^{film} 5.70 μ (C=O), 7.28 μ (CH_3), 8.18 μ (acetate C–O–C), 9–10 μ (sugar C–O–C); $[\alpha]_D^{23.5}$ +51° (1% in chloroform).

Chang and Lythgoe (1950) reported $[\alpha]_D^{19}$ +56° (2.1% in alcohol).

*6-Amino-2-chloro-9-(3′,5′-O-isopropylidene-β-*D*-xylofuranosyl)-9H-purine (IV).*—A mixture of 21.0 g (66.3 mM) of xylose tetraacetate and 12.5 g (66.6 mM) of 2,6-dichloropurine (I) was heated at 130° under a water aspirator vacuum for 2–3 minutes with stirring to mix and dry the reactants. After vigorous bubbling had ceased the mixture was cooled to room temperature, the vacuum was released, and 320 mg of *p*-toluenesulfonic acid monohydrate was added. The flask was re-evacuated using a water aspirator and the mixture was heated at 130° with stirring, complete solution resulting after 5–10 minutes. The reaction was heated at 130° for 20–25 minutes, then cooled to room temperature, and the residue was dissolved in 40 ml of chloroform. A small amount of unreacted 2,6-dichloropurine was removed by filtration. The chloroform filtrate was washed with 50 ml of saturated aqueous sodium bicarbonate and 30 ml of water, then dried over magnesium sulfate and evaporated to dryness *in vacuo* to a light brown sirup. The sirup was dissolved in 60 ml of methanol. The methanol solution was cooled to 0° and saturated with ammonia. The ammoniacal solution was left for 36–40 hours at room temperature in a sealed Parr bomb. At the end of this time the bomb was cooled to 0° and the reaction solution was evaporated to dryness *in vacuo*. The residue was partitioned between 200 ml each of water and chloroform. The water layer was evaporated to dryness *in vacuo* in a 500-ml round-bottom flask. The last traces of water were removed by heating at 1 mm Hg in a water bath at about 60° for 3–4 hours.

The residue from the water layer, crude compound III, was stirred with 200 ml of acetone and 6 ml of ethanesulfonic acid (the ethanesulfonic acid was added dropwise with stirring) for 18 hours. The acetone solution was decanted from the sludge and poured into 400 ml of saturated aqueous sodium bicarbonate. The aqueous acetone solution was concentrated to one-half volume to remove the acetone. The resulting precipitate was filtered and dried to give 7.9 g of product as a pale yellow solid, mp 145–150°, which was of satisfactory quality for the next step. The analytical sample recrystallized from 50% aqueous ethanol had mp 147–150°; λ_{max}^{Nujol} 6.0, 6.25, 6.37 μ (purine ring); 8.35, 8,42, 11.75 μ (isopropylidene); $\lambda_{max}^{pH\,1}$ 265 mμ (ε, 13,800); $\lambda_{max}^{pH7.13}$ 264 mμ (ε, 14,700).

Anal. Calcd. for $C_{13}H_{16}ClN_5O_4 \cdot 0.4\ H_2O$: C, 44.7; H, 4.85; Cl, 10.2; N, 20.1. Found: C, 44.4; H, 5.37; Cl, 10.0; N, 20.1.

Hydrogenation of compound IV at atmospheric pressure with 5% palladium-on-charcoal and magnesium oxide in 2-methoxyethanol gave 9-(3′,5′-*O*-isopropylidene-β-D-xylofuranosyl)-adenine which was identical with authentic material prepared by a different method (Reist *et al.*, 1962).

*6-Amino-2-chloro-9-(3′,5′-O-isopropylidene-2′-O-methylsulfonyl-β-*D*-xylofuranosyl)-9H-purine (V).*—A solu-

tion of 7.9 g of crude 6-amino-2-chloro-9-(3′,5′-*O*-isopropylidene-β-D-xylofuranosyl)-9H-purine (IV) (dried at 0.1 mm Hg at 56° for 5 hours) in 140 ml of dry pyridine was cooled to 5° in an ice bath, then 8.0 ml of methanesulfonyl chloride was added dropwise with stirring. After the addition was complete, the reaction was stored at room temperature for 48 hours. At the end of this time, the reaction was cooled to 0°, then the excess methanesulfonyl chloride was decomposed by the cautious addition of 0.5 ml of water. The decomposed reaction mixture was stirred for 0.5 hour, then partitioned between 400 ml each of chloroform and saturated aqueous sodium bicarbonate. The chloroform layer was washed with 100 ml of water, then evaporated to dryness *in vacuo* to give 9.2 g of product as a brown solid which showed strong sulfonate bands at 7.3, 8.5, and 12.4 μ in the infrared and was satisfactory for the next step. This product had R_{Ad} 0.0 in solvent B. The starting material had R_{Ad} 1.3 in this same system.

Recrystallization from 95% ethanol gave the analytical sample, mp 142–145°; λ_{max}^{Nujol} 6.01, 6.25, 6.35 μ (purine); 8.45 μ (OSO_2); 11.83 (isopropylidene); $\lambda_{max}^{pH\ 1}$ 261 mμ (ε, 19,700); $\lambda_{max}^{pH\ 7}$ 261 mμ (ε, 20,800).

Anal. Calcd. for $C_{14}H_{18}ClN_5O_6S$: C, 40.1; H, 4.33; Cl, 8.44; N, 16.7. Found: C, 39.7; H, 4.90; Cl, 8.51; N, 16.4.

6-Amino-2-chloro-9-(2′-O-methylsulfonyl-β-D-xylofuranosyl)-9H-purine (VI).—A solution of 9.2 g of crude 6-amino-2-chloro-9-(3′,5′-*O*-isopropylidene-2′-*O*-methylsulfonyl-β-D-xylofuranosyl)-9H-purine (V) in 100 ml of 90% aqueous acetic acid was heated on a steam bath for 7 hours; then it was evaporated to dryness *in vacuo* to give 8.6 g of product as a brown sirup which crystallized on standing and which was satisfactory for the next step. Two 20-ml portions of water were added and evaporated at 50–60° at 1 mm Hg to ensure complete removal of all the acetic acid. Paper chromatography in solvent B showed one spot at R_{Ad} 1.2.

Recrystallization from methanol, then twice from water, gave white crystals, mp 215.5–216.0° dec.; λ_{max}^{Nujol} 3.01, 3.16 μ (OH, NH); 6.00, 6.25 μ (purine); 8.45 (OSO_2).

Anal. Calcd. for $C_{11}H_{14}ClN_5O_6S$: C, 34.9; H, 3.74; Cl, 9.36; N, 18.4; S, 8.43. Found: C, 35.3; H, 4.29; Cl, 8.90; N, 18.1; S, 7.95.

6-Amino-2-chloro-9-(2′,3′-anhydro-β-D-lyxofuranosyl)-9H-purine (VII).—A solution of 8.0 g of dry 6-amino-2-chloro-9-(2′-*O*-methylsulfonyl-β-D-xylofuranosyl)-9H-purine (VI) in 150 ml of absolute methanol which contained 1.5 g of sodium methoxide was heated at reflux for 12 minutes. (At the end of the reflux period the *p*H of the solution was checked. If the solution was no longer strongly basic, more sodium methoxide was added, and an extra reflux period was required.) The reaction was cooled, then neutralized with glacial acetic acid and evaporated to dryness *in vacuo* to give crude compound VII as a brown solid. Trituration with 20 ml of water gave 7.0 g of brown-colored crystals, mp 225° dec., which were of satisfactory quality for the next step.

Paper chromatography in solvent B showed one spot at R_{Ad} 1.10, easily distinguishable from compound VI. In solvent A the product had R_{Ad} 1.5 (starting material had R_{Ad} 1.3).

Two recrystallizations from 95% ethanol gave the analytical sample, mp 223.5–225.0°; λ_{max}^{Nujol} 2.90, 3.00, 3.20 μ (OH, NH); 6.00, 6.07, 6.25, 6.32 μ (purine); 8.52, 11.22 μ (epoxide).

Anal. Calcd. for $C_{10}H_{10}ClN_5O_3$: C, 42.4; H, 3.56; Cl, 12.5; N, 24.7. Found: C, 42.6; H, 3.96; Cl, 12.3; N, 24.3.

6-Amino-2-chloro-9-β-D-arabinofuranosyl-9H-purine (VIII).—A mixture of 7.0 g of anhydrous sodium acetate and 7.0 g of 6-amino-2-chloro-9-(2′,3′-anhydro-β-D-lyxofuranosyl)-9H-purine (VII) in 280 ml of 95% aqueous *N,N*-dimethylformamide was heated with stirring at 150° for 7 hours, then was cooled and evaporated to dryness *in vacuo*. The residue was dissolved in 15 ml of water. The aqueous solution was cooled and scratched to give 3.13 g of product, mp 238–242° dec., in three crops.

The product had R_{Ad} 0.88 in solvent A and R_{Ad} 1.27 in solvent B. Two recrystallizations from water gave white crystals; mp 239.0–240.5° dec.; λ_{max}^{Nujol} 2.90, 2.98, 3.22 μ (OH, NH_2); 6.00, 6.23, 6.37 μ (purine); $\lambda_{max}^{pH\ 1}$ 265 mμ (ε, 14,000); $\lambda_{max}^{pH\ 7}$ 264 mμ (ε, 15,000); $\lambda_{max}^{pH\ 13}$ 265 mμ (ε, 15,200).

Anal. Calcd. for $C_{10}H_{12}ClN_5O_4 \cdot 1/4\ H_2O$: C, 39.3; H, 4.12; Cl, 11.6; N, 22.9. Found: C, 39.5; H, 4.26; Cl, 11.3; N, 22.5.

Hydrogenation of compound VIII at atmospheric pressure with 5% palladium-on-charcoal and triethylamine in 2-methoxyethanol gave 9-β-D-arabinofuranosyladenine which was identical with authentic 9-β-D-arabinofuranosyladenine (Reist *et al.*, 1962) in solvents A, B, and C and which showed no melting point depression with authentic 9-β-D-arabinofuranosyladenine.

2-Chloro-6-hydroxy-9-β-D-arabinofuranosyl-9H-purine (IX).—A solution of 491 mg of 6-amino-2-chloro-9-β-D-arabinofuranosyl-9H-purine (VIII) in 12.5 ml of glacial acetic acid was effected by gentle warming to about 50°. The solution was cooled to room temperature and 500 mg of sodium nitrite was added in portions with stirring. After about 1 hour inorganic salts began to precipitate. The reaction was left at room temperature for 24 hours, then an additional 100 mg of sodium nitrite was added and the reaction was left at room temperature for a further 24 hours. The reaction was evaporated to dryness *in vacuo* and the residue was triturated with 5 ml of cold water to give 121 mg of product as a white solid, mp 195–200° dec.

A second crop could be obtained via the lead salt. The filtrate (5 ml) was made alkaline with 2 ml of concentrated aqueous ammonia, then 2 ml of saturated aqueous lead acetate was added. There was an immediate heavy precipitation of the lead salt. The precipitate was filtered *in vacuo* and washed with dilute aqueous ammonia then dissolved in 10–15 ml of 50% aqueous acetic acid. Gaseous hydrogen sulfide was passed through the acetic acid solution for 20 minutes, and the precipitated lead sulfide was removed by vacuum filtration through a Celite pad. The colorless filtrate was evaporated to dryness *in vacuo* to give 189 mg of a viscous yellow oil. Crystallization of this oil from 2 ml of water gave an additional 50 mg of product, mp 195–197° dec.

The analytical sample had mp 195–197° dec.; λ_{max}^{Nujol} 2.96, 3.04, 3.18 μ (OH, NH); 5.85, 6.31, 6.42 μ (purine); $\lambda_{max}^{pH\ 1}$ 252 mμ (ε, 10,500); $\lambda_{max}^{pH\ 7,13}$ 257 mμ (ε, 13,100).

Anal. Calcd. for $C_{10}H_{11}ClN_4O_5 \cdot 0.33\ H_2O$: C, 38.9; H, 3.82; Cl, 11.5; N, 18.1. Found: C, 39.1; H, 3.74; Cl, 11.5; N, 17.8.

9-β-D-Arabinofuranosylguanine (X).—To 20 ml of methanol, which had been saturated with ammonia at 0°, in a Parr bomb, was added 120 mg of recrystallized 2-chloro-6-hydroxy-9-β-D-arabinofuranosyl-9H-purine (IX). The reaction was heated at 150–160° for 7 hours, then cooled and evaporated to dryness *in vacuo*. The resulting solid was purified via the lead salt in the manner described for the preparation of 2-chloro-6-hydroxy-9-β-D-arabinofuranosyl-9H-purine (IX) to give 97 mg of a white solid which was homogeneous on

paper chromatography with R_{Ad} 1.92 in solvent B and R_{Ad} 0.45 in solvent C.

Recrystallization from 2.5 ml of water gave 54 mg of white needles, mp > 300° (darkens from 265°), and a second recrystallization gave the analytical sample, mp > 300° (darkens from 265°); $[\alpha]_D^{24}$ +28° (0.25% in water); $\lambda_{max}^{pH\,1}$ 256 mμ (ε, 12,600); $\lambda_{max}^{pH\,7}$ 252 mμ (ε, 14,000); $\lambda_{max}^{pH\,13}$ 256 mμ (ε, 11,600), 265 mμ (ε, 11,800).

Anal. Calcd. for $C_{10}H_{13}N_5O_5 \cdot H_2O$: C, 39.9; H, 5.02; N, 23.2. Found: C, 40.0; H, 5.02; N, 23.3.

Guanosine has $\lambda_{max}^{pH\,1}$ 256 mμ (ε, 12,200); $\lambda_{max}^{pH\,11.3}$ 258–266 mμ (ε, 11,300) (Beaven *et al.*, 1955).

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The Stability Constants of Metal-Adenine Nucleotide Complexes

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Received July 18, 1963

Values of 73,000 M^{-1} and 4,000 M^{-1} have been obtained for the stability constants of $MgATP^{2-}$ and $MgADP^{-}$, respectively, in 0.1 M *N*-ethylmorpholine buffer at 30°. In addition, reasonable confirmation of the value for $MgATP^{2-}$ has been obtained from *p*H titration data, using both an approximation method and a program written for an IBM 1620 computer. Values for the stability constants of $CaATP^{2-}$ by *p*H titration and spectrophotometry and $CaADP^{-}$ and $MnADP^{-}$ by spectrophotometry have been obtained. Results for $NaATP^{3-}$, $KATP^{3-}$, Mg-phosphocreatine, and Mg-phosphoarginine are also presented.

Divalent metal ions are required as activators (and also sometimes act as inhibitors) of many of the enzymic reactions involved in the metabolism of nucleotide phosphates. These ions interact non-enzymically with nucleotide phosphates to form the respective metal complexes, so that a knowledge of the stability constants of these complexes is necessary for the interpretation of enzyme kinetic and thermodynamic studies. This information may, in fact, be a prerequisite to many such investigations (Morrison *et al.*, 1961).

Values, varying widely in magnitude, have been given for the stability constants of $MgADP^{-}$ and $MgATP^{2-}$ (Table I; Bock, 1960). Reasons for these variations may not be obvious to an enzymologist wishing to decide on the value he should use.

It is necessary to distinguish between absolute and apparent values for these stability constants. The absolute values, usually obtained from *p*H titration data, are true constants, independent of *p*H, whereas an "apparent" value relates only to a particular *p*H, and for the reaction

$$M + L \rightleftharpoons ML$$

may be defined as

$$K_{app} = \frac{[ML]}{[M][L]_{T'}}$$

where $[L]_{T'}$ is the sum of the uncomplexed forms of L at that *p*H. Under certain conditions the "apparent" and absolute values may be approximately the same. However, where results are to apply to enzyme experiments at constant *p*H it is convenient to use the relevant "apparent" value (which can be obtained from the absolute value provided *p*H and pK_a values are known). The magnitude of both the absolute and the "apparent" values will vary with such experimental variables as the nature of the supporting medium, ionic strength, and temperature, and it is useful to consider briefly the effect of these factors. Some of these points are treated in more detail later in the text.

The application of classical potentiometric titration techniques to stability-constant determinations involving polyanionic molecules such as ATP can lead to mathematical expressions of such complexity that they are incapable of exact solution (see below). For this reason, workers (e.g., Martell and Schwarzenbach, 1956; Smith and Alberty, 1956a; Nanninga, 1961) have used various simplifications which appear to be of doubtful validity (see Bock, 1960).

The principal methods used for determinations of "apparent" constants have been dependent upon competition between ligands (e.g., Walaas, 1958; Burton, 1959; Nanninga, 1961), the required *p*H being maintained by a suitable buffer. Thus in making

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