REFERENCES

1 F. F. DAVIS AND F. W. ALLEN, *J. Biol. Chem.*, 227 (1957) 907.
2 C. T. YU AND F. W. ALLEN, *Biochim. Biophys. Acta*, 32 (1959) 393.
3 J. P. SCANNELL, A. M. CRESTFIELD AND F. W. ALLEN, *Biochim. Biophys. Acta*, 32 (1959) 406.
4 W. E. COHN, *Biochim. Biophys. Acta*, 32 (1959) 569.
5 H. MCILWAIN, *J. Gen. Microbiol.*, 2 (1948) 288.
6 R. MARKHAM AND J. D. SMITH, *Biochem. J.*, 45 (1949) 216.
7 R. MARKHAM AND J. D. SMITH, *Biochem. J.*, 45 (1949) 294.
8 K. C. SMITH AND F. W. ALLEN, *J. Am. Chem. Soc.*, 75 (1953) 2131.
9 E. S. CANELLAKIS, *J. Biol. Chem.*, 221 (1956) 315.
10 C. E. CARTER, *J. Am. Chem. Soc.*, 73 (1951) 1508.
11 L. M. PAEGE AND F. SCHLENK, *Arch. Biochem.*, 28 (1950) 348.
12 L. A. MANSON AND J. O. LAMPEN, *J. Biol. Chem.*, 191 (1951) 95.

Biochim. Biophys. Acta, 44 (1960) 224–232

NON-ENZYMIC FORMATION OF DIAZOTIZABLE AMINE FROM RIBOSYLADENINE

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(Received March 5th, 1960)

SUMMARY

Adenosine is cleaved non-enzymically in the presence of ketopentose, Cu^{++}, and pyrophosphate at 70° to yield small amounts of 5-amino-1-ribosyl imidazole. A similar diazotizable amine may be produced non-enzymically from ATP under conditions found in biological experiments. The preparation and characterization of the imidazole derived from adenosine are described. Two additional adenosine derivatives which are produced during the reaction have been partially characterized as 6-amino substituted compounds.

INTRODUCTION

ATP in the presence of pentose phosphate undergoes a non-enzymic degradation which leads to the formation of small amounts of diazotizable amine. This reaction may occur as an artefact in biological experimentation and illustrates some conditions which labilize the pyrimidine moiety of purine ring systems. The production of diazotizable amine from ATP is attributable to the ketopentose content of pentose phosphate preparations and requires the presence of cupric ion. While ketopentose phosphate appeared to be the most reactive carbohydrate component, other compounds of a ketose character, such as fructose and glycol aldehyde, promote formation

Abbreviations: ATP, adenosine triphosphate; Tris tris(hydroxymethyl)aminomethane.

of the amine. Degradation of the purine to diazotizable amine at 37° was most extensive when ATP was employed; adenosine, adenosine monophosphate and diphosphate were less effective precursors of amine and these reactants required higher temperature and the presence of pyrophosphate. Neither adenine nor the phosphorylated ribosyl derivatives of other purines yielded diazotizable amine. The reaction most extensively studied, because of the availability of starting materials, has been that involving adenosine, fructose and cupric ion in pyrophosphate buffer. The diazotizable amine product has been tentatively identified, on the basis of its absorption spectra, chromatographic behavior, and color reactions, as 5-amino-1-ribosylimidazole. Two adenosine derivatives, both of which are tentatively considered to be 6-amino substituted compounds, are produced during the reaction and have been chromatographically separated and partially characterized.

EXPERIMENTAL

Materials

Adenosine triphosphate was obtained from Pabst and adenosine from Schwarz Laboratories, Inc. Ribose-5-phosphate was prepared from the barium salt (Nutritional Biochemical Corporation) by passing the acidified solution through a column of Dowex-50 H^+ resin. 5-phosphoribosylpyrophosphate was prepared enzymically, purified and assayed according to the methods described by KORNBERG *et al.*[1]. Ribose 5-phosphate isomerase, prepared from spinach and purified through the cysteine dialysis step, was employed for the synthesis of ribulose-5-phosphate and the latter compound was purified by column chromatography[2,3]. Prostatic phosphatase was prepared by dialyzing freshly homogenized tissue against distilled water for 16 h[4]. Ribulose and xylulose were prepared by epimerization of the aldose in pyridine[5].

Analytical methods

Ketopentose was determined by the cysteine–carbazole reaction[6], diazotizable amine by the method of BRATTON AND MARSHALL[7], imidazole by the KOESSLER-HANKE modification of the PAULY reaction[8], and ribose by the method of MEJBAUM[9]. Periodate titrations were performed according to DYER[10]. Sugars were identified, after dephosphorylation with prostatic enzyme when indicated, by chromatography in phenol–water (9:1) (see ref. 2).

Diazotizable amine was detected on paper by a modification of the method of SMITH[11] in which a saturated solution in absolute alcohol of N(1-naphthyl) ethylenediamine dihydrochloride was substituted for the Ekman reagent. Ribose was detected by periodate dip[12], reducing sugar by aniline phthalate[13] and ketose by a trichloracetic–orcinol spray[14]. Imidazole was detected by means of the PAULY reaction[15].

Reactions with ATP

At 37° in Tris–HCl buffer (0.5 *M*, pH 7.0), ATP and ribose-5-phosphate react in the absence of tissue extracts to yield a trace amount of amine which was detected by the BRATTON-MARSHALL reaction. When cupric ion was added to the mixture the yield of amine was increased 10-fold. The optimal concentration of cupric ion was a function of the concentration of ATP and pentose phosphate employed. In incubation mixtures of 1 ml containing 10 μmoles ATP and 16 μmoles pentose

phosphate, optimal cupric ion was 2.5 μmoles. Purification of ATP through anion exchange chromatography[16] did not alter its reactivity for amine production in this system. Bromine oxidation of ribose phosphate, which degrades aldopentose compounds[3], did not decrease the yield of amine. Since commercial ribose-5-phosphate preparations contain ketopentose which survives the latter treatment, enzymically synthesized ribulose-5-phosphate was used in the reaction mixture. As shown in Table I, treatment of ribose-5-phosphate with spinach epimerase increased the content of carbazole reacting material (ketopentose) 3-fold and incubation of this mixture with ATP and Cu^{++} gave an approx. 4-fold increase of amine. Dephosphorylation of the pentose mixture with prostatic phosphatase prior to incubation with ATP and Cu^{++} resulted in 50 % lower yields of amine. When partially purified ribulose and xylulose, synthetically prepared by pyridine epimerization, were employed, the yield of amine was lower than that resulting from incubation with enzymically synthesized ketopentose of comparable concentration (based on carbazole reacting material). This may be due to trace contamination with pyridine in the synthetic product which interferes with the interaction of Cu^{++} and purine. 5-phosphoribosyl-1-pyrophosphate did not react to yield diazotizable amine, nor did glucose, glucose-1-phosphate, glucose-6-phosphate, ribose, xylose or arabinose. At 37° fructose gave a

TABLE I

EFFECT OF TREATING RIBOSE-5-PHOSPHATE WITH RIBOSE-5-PHOSPHATE ISOMERASE

Reaction mixture contained in 1 ml of 0.025 *M* Tris buffer, pH 7.0: ATP 4.7 μmoles, $CuCl_2$ 0.1 μmole and ribose-5-phosphate 44 μmoles. The mixture was incubated at 37° in an atmosphere of air for 3 h.

	Cysteine-carbazole reaction Optical density/ml	*Bratton Marshall reaction Optical density/ml*
Pentose-phosphate	700	115
Treated with isomerase	2,050	400
Then phosphatase	2,000	214

low yield of amine which was increased by incubating the reaction mixture at 80°. The relationship of ketopentose concentration to amine production was studied under the conditions described above. Amine production was optimal at 37°, pH 7 in Tris buffer (0.025 *M*) when the ratio of ATP, ketopentose-phosphate and Cu^{++} was 15:4:2.5. Amounts of ketopentose in excess of 0.004 *M* produced no further increase in amine production; high concentrations of sugars were inhibitory. The proportion of Cu^{++} ion necessary for optimal yield of amine was dependent upon the relative proportion and amount of the other two reactants and the buffer. In general, cupric ion concentrations in molar excess with respect to the carbohydrate strongly inhibited the reaction. The requirement for cupric ion could not be replaced by Cu^{+}, Mg^{++}, Mn^{++}, Fe^{++}, Fe^{+++}, Co^{+}, Zn^{++} or Ca^{++}. The reaction was completely inhibited by 10^{-3} *M* ethylenediaminetetraacetic acid. When mixtures were incubated anaerobically in the presence of N_2 the yield of amine was greatly decreased. An optimal yield of amine was obtained by shaking the mixture in air throughout the incubation period. However, the yield was not increased by an atmosphere of pure O_2.

The amount of diazotizable amine in the reaction mixtures increased slowly

during the first 3 h of incubation at 37°, then declined. During this period ketopentose concentration was continually decreasing whether all the reactants were present or not, and addition of ketopentose at 30 min intervals did not increase the yield. In 0.1 *N* acid or alkali the arylamine was rapidly destroyed at room temperature. It was adsorbed on Dowex-1-formate but could not be recovered with formic acid or buffered formate. On paper chromatography the amine moved in several solvent systems as a band which partially overlapped ATP.

The yield of BRATTON-MARSHALL reacting amine from ATP under optimal conditions was about 1 %, based on a molar extinction coefficient at 504 mμ of 24,000 for the colored product of the diazotized arylamine. This value is approximately that (24,600) reported for aminoimidazoleribosylphosphate at 500 mμ (see ref. 17) and for aminoimidazolecarboxamideribosylphosphate (26,400) at 540 mμ (see ref. 18). The absorption maximum of the compound formed in the BRATTON-MARSHALL reaction with the amine derived from ATP was 504 mμ.

Reaction with adenosine

Chromatographic evidence indicated that the arylamine derived from ATP was a ribosylpyrophosphate and that it was more labile than the ribosylarylamine which could be produced under analogous conditions from adenosine. The conditions for amine production from adenosine, fructose and cupric ion were similar to those found optimal for ATP and ketopentose except that pyrophosphate was required and it was necessary to increase the temperature of the reaction mixture to 70°. The optimal pH was 7.6 to 8.0. The following conditions were employed. To 100 ml of distilled water 5 mmoles of adenosine, 5 mmoles of $Na_4P_2O_4$ and 3 mmoles of $CuCl_2$ were added and the mixture was heated at 100° for 20 min. This solution was added slowly with stirring over a period of 30 min to a solution of 2 mmoles of fructose in 50 ml distilled water at 70°. During this time a flocculent precipitate formed and the mixture became a pale yellow color. Incubation was continued at 70° and the mixture was vigorously aerated while the volume was maintained at 150 ml by frequent addition of distilled water. In the absence of aeration the yield of amine was decreased over 70 %. Continued amine production ceased after approx. 1.5 h during which time the precipitate disappeared and the mixture became brown. The sample was then concentrated under reduced pressure to 50 ml and allowed to stand at 4° for 16 h. The heavy precipitate which formed consisted mainly of adenosine. It was removed by filtration. About 90 % of the amine in the original solution

TABLE II

R_F IN SOLVENT SYSTEMS

1, *n*-propanol–0.3 *N* NH_4OH (3:1), Whatman No. 3, descending. 2, *n*-propanol–water (3:1), Whatman No. 1, ascending. 3, *n*-propanol–1 *N* acetic acid (3:1), Whatman No. 1 ascending. 4, *n*-propanol–0.2 N NH_4OH (3:1), Whatman No. 1 ascending. 5, *n*-butanol–glacial acetic acid–water (2:2:1), Whatman No. 1 descending.

	1	*2*	*3*	*4*	*5*
Compound I (arylamine)	0.65	0.43	0.38	0.42	0.42
Compound II	0.51	0.29	0.35	0.29	0.41
Compound III	0.39	0.20	0.21	0.18	0.33

was recovered in the filtrate. 50 ml of water were added to the filtrate and this solution was passed through a column of Dowex-1-formate, 3.2 $cm^2 \times 16$ cm. The effluent was tested for arylamine at frequent intervals and after the accumulation of about 40 ml (the retention volume of the column) when the test became positive, collection of the sample was begun. The remaining original volume was collected and the column washed with 0.005 *M* sodium pyrophosphate (about 40 ml) until arylamine concentration began to decrease. These effluent solutions, which contained essentially all the arylamine applied and fructose, but virtually no cupric ion or adenosine, were combined, diluted to 200 ml and adjusted to pH 4 with 1 *M* formic acid. This solution was then passed through a Dowex-50-Na^+ column (2.6 $cm^2 \times 7$ cm) and the column washed with 20 ml of water. The arylamine which was adsorbed to the column was recovered in highest yield by eluting with 0.03 *M* NH_4OH and collecting the first 15 ml of effluent after the pH rose above 6.5. The lability of the amine did not permit gradient elution with salt or acid. If the column operations were conducted rapidly and the solutions chilled in ice, the overall recovery of arylamine through this step was about 80 %.

Further purification of arylamine was achieved by paper chromatography. The ammonium hydroxide eluate was concentrated by vacuum distillation to 3–4 ml and applied to a 24 × 18 cm sheet of Whatman No. 3 paper. Descending chromatograms employing *n*-propanol–0.3 *N* NH_4OH (3:1) were run for 16 h. After the papers were dried, the u.v. absorbing bands were located, the paper was cut in corresponding strips and the strips were eluted with a small volume of distilled water. Three principal components were separated in this system as shown in Table II (adenosine has an R_F of 0.72 in solvent 1).

Compound I (diazotizable amine)

In the BRATTON-MARSHALL reaction this compound yielded a salmon-pink color with maximum absorption at 520 mμ in 5 % trichloracetic acid or 4 % perchloric acid. The amine was not acetylated by treatment with acetic anhydride. In several solvent systems (Table II) the compound gave a single spot which weakly adsorbed u.v. light, gave a strong arylamine reaction, a permanent yellow color in the PAULY reaction, and a positive periodate and ninhydrin reaction. When an extinction coefficient of 24,000 for the arylamine reaction product was employed[17], and ribose determined by the orcinol reaction, a ratio of arylamine to ribose of 0.8 was found. The ratio of less than unity is attributed to the lability of the arylamine during the process of separation and recovery from paper. On standing at room temperature at neutral pH the amount of BRATTON-MARSHALL reacting material decreased and the solution became yellow over a period of a few hours. Treatment with 0.1 *N* HCl at 100° for 10 min destroyed the arylamine. The u.v. absorption spectrum of the freshly prepared compound showed no maximum above 230 mμ in acid, neutral or alkaline solution. Attempts to convert the arylamine to a ureido derivative, which could be isolated as the picrate, were unsuccessful, probably because of the small amounts of compound employed and its lability. Based on the BRATTON-MARSHALL reaction the amount of chromatographically purified arylamine derived from 1.8 g of adenosine was 10 mg or 0.5 %; on the basis of ribose determined in the orcinol reaction the yield was 0.63 %.

Compounds (II) and (III)

Chromatographically isolated compounds (II) and (III) reacted with periodate on paper and gave positive orcinol reactions for ribose. The yield of these compounds from adenosine expressed as μmoles ribose determined in the orcinol reaction was: Compound II, 2.7%, and compound III, 1.3%. Hydrolysis of aliquots of these compounds (II and III) in 0.1 *N* NaOH at 100° for 5.5 h degraded both compounds to one u.v. absorbing component; this was identified as adenine on the basis of spectra and migration with the same R_F as adenine in several solvent systems; namely, in *n*-butanol–glacial acetic acid–water (2:1:1), in isoamyl alcohol saturated with 5% Na_2HPO_4 and in isopropanol–1.2 *N* NH_4OH (5:3:1). The amount of adenine as determined by u.v. spectrophotometry on the eluate of the compounds separated by paper chromatography was approximately equimolar with the amount of ribose as determined by the orcinol reaction on a corresponding aliquot. When the compounds (II and III) were hydrolyzed in 2 *N* acetic acid at 100° for 2 h and the hydrolysate was chromatographed in *n*-butanol–glacial acetic acid–water (2:1:1) the original compounds were no longer present. Each parent compound gave rise to two new components; a carbohydrate with the R_F of ribose and a single u.v. absorbing spot with an R_F of 0.50 derived from (II) and 0.53 derived from (III). Hydrolysis in 0.1 *N* or 1.0 *N* HCl for 10 to 60 min at 100° degraded the compounds (II and III) to several poorly resolved fluorescent and u.v. absorbing products, none of which gave positive ninhydrin, Pauly, or BRATTON-MARSHALL tests.

The u.v. absorption spectra of the compounds (II and III) are shown in Figs. 1 and 2. Both compounds show hyperchromic shifts in acid and bathochromic shifts

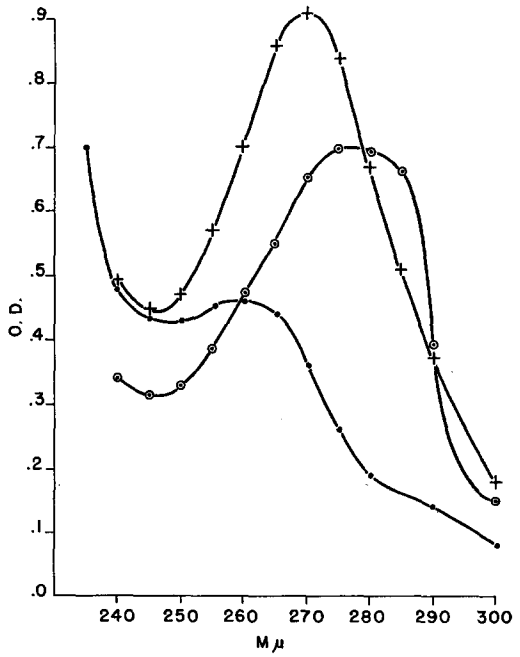

Fig. 1. The u.v. absorption spectra of compound II. +—+, in 0.1 *N* HCl; ●—●, in 0.05 *M* potassium phosphate, pH 7.4; ⊙—⊙, in 0.1 *N* NaOH.

in alkali. Dissociations were found at pH 3.7 and 12.0 for both compounds by plotting the spectral change at 270 mμ as a function of pH. The u.v. absorption spectra of the compounds (II and III) were not changed by treatment with nitrous acid under

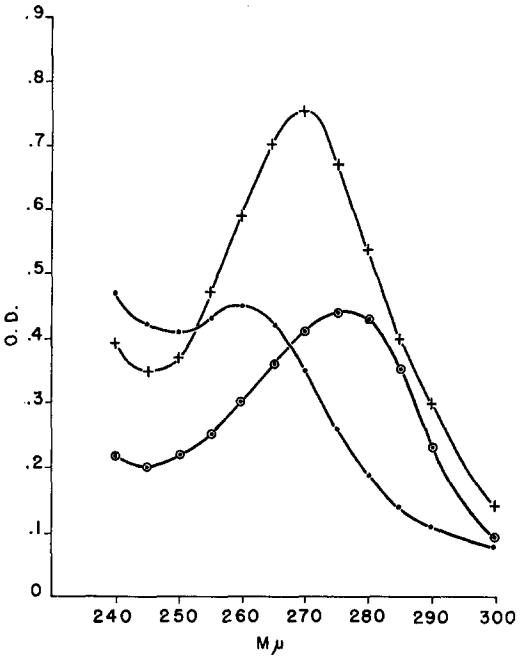

Fig. 2. The u.v. absorption spectra of compound III. +—+, in 0.1 *N* HCl; ●—●, in 0.05 *M* potassium phosphate, pH 7.4; ⊙—⊙, in 0.1 *N* NaOH.

conditions which deaminate adenosinemonophosphate or diphosphopyridine nucleotide[19]. The u.v. absorbing derivative of the compounds (II and III) obtained by chromatographic separation and elution of the products of 2 *N* acetic acid hydrolysis contained no ribose and exhibited u.v. absorption characteristics similar to the parent compound except that the maxima in acid and alkali were shifted 5 mμ to a shorter wavelength. The u.v.-absorbing component of compound II did not consume periodate, as determined by arsenite titration, and did not react with periodate on paper chromatograms. The aglycone of compound III slowly consumed periodate in solution and reacted with periodate on paper to yield a dark blue spot after the benzidine spray. The stoichiometry of the periodate titration is not established. However, if the extinction coefficient at pH 7.0 of III-aglycone is assumed to be the same as that for adenine, approx. 1 μmole of periodate is consumed/μmole of purine. Since the compound contains no ribose this finding indicates that a group reacting with periodate is substituted on the ring or the amino group of the adenine moiety.

DISCUSSION

The arylamine derived from adenosine in a reaction with fructose, Cu^{++} and pyrophosphate gives a Pauly test for imidazole, a BRATTON-MARSHALL reaction for

non-acetylatable arylamine (maximum 520 mμ), exhibits no u.v. absorption maxima above 230 mμ, and contains approximately equimolar amounts of arylamine and ribose. This evidence supports the proposed structure of 5-amino-1-ribosylimidazole. The analogous reaction of ATP, ketopentose phosphate, and Cu^{++} is believed to produce the pyrophosphate derivative of 5-aminoimidazoleribosyl-5'-phosphate. However, this product has not been chromatographically purified and characterized and the proposed identity is supported only by analogy.

The two adenosine derivatives, which may be intermediates or by-products in the reaction leading to arylamine production from adenosine and fructose, have substituents which are cleaved in 0.1 *N* NaOH to yield adenine. These compounds show pronounced u.v. absorption shifts in acid and alkali and treatment with nitrous acid does not change their spectrophotometric characteristics. The findings are consistent with substitution on the 6-amino position of adenosine. The two derivatives apparently differ from each other with respect to the character of the substituent group. In the aglycone of compound III, the substituent reacts with periodate, while this is not the case with compound II. Both substituents may be low molecular weight fragments derived from fructose, but the nature of the groups is not known.

The reaction leading to ribosylaminoimidazole formation from adenosine and its derivatives is complex and probably involves equilibria which permit the accumulation of only small amounts of reactive intermediates. Copper may participate in several phases of the reaction. It is significant that adenine, which chelates strongly with cupric ion and simultaneously loses a proton[20], does not give rise to amine; a ribosyl substituent on N 9 of adenine is essential for the reaction. Since ATP does not require additional pyrophosphate to serve as an arylamine precursor, while adenosine does, it would appear that pyrophosphate enters into the formation of the cupric chelate. A formulation of the reaction of Mg^{++} with the 6-amino group, N-7, and the pyrophosphate group of ATP has been discussed by SZENT-GYÖRGY[21].

The ketose configuration $-\overset{O}{\overset{\|}{C}}-\overset{OH}{\overset{|}{C}}-H_2$, which is essential to the carbohydrate component of the degradative reaction, may add to the chelated purine at N-1, the 6-amino, or at both positions. Previous studies have demonstrated that purines substituted in these positions exhibit characteristic lability. SHAW[22] found that benzyl substitution on N-1 of inosine rendered the purine labile to alkaline hydrolysis with the resultant formation of ribosylaminoimidazole benzylcarboxamide. In BROWN'S laboratory[23] it was shown that adenine N-1 oxide cleaved in acid far more readily than adenine to yield aminoimidazolecarboxamide. N-succinoadenine, a compound substituted on the 6-amino group, was also found to be degraded in acid to aminoimidazolecarboxamide[24], a reaction which probably involves an attack of the substituent carboxyl group upon N-1 of the purine ring.

While ribosylaminoimidazole has been detected in the culture fluid or bacteria and molds[25–27], conditions for the chemical degradation of adenosine to the ribosylaminoimidazole have not been reported. The evidence cited above[22–24] indicates that the carboxamide group of aminoimidazolecarboxamide is resistant to cleavage in acid or alkali. Therefore, a mechanism for degradation for adenosine to aminoimidazole probably involves removal or complexing of both N-1 and the 6-amino group in such a manner as to yield the labile 5-amino-4-carboxyimidazole riboside which

decarboxylates to the amino compound. The dependence of the overall reaction upon aerobic conditions implies an oxidative step in the sequence.

An understanding of the mechanism of enzyme reactions which open the pyrimidine portion of purine ring systems may be developed by further study of the chemical degradations described.

REFERENCES

[1] A. KORNBERG, I. LIEBERMAN AND E. S. SIMS, *J. Biol. Chem.*, 215 (1955) 393.
[2] M. TABACHNICK, P. A. SRERE, J. COOPER AND E. RACKER, *Arch. Biochem. Biophys.*, 74 (1958) 315.
[3] P. A. SRERE, J. COOPER, M. TABACHNICK AND E. RACKER, *Arch. Biochem. Biophys.*, 74 (1958) 295.
[4] SCHMIDT, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. II, Academic Press, Inc., New York, New York, 1955, p. 523.
[5] P. A. LEVENE AND R. S. TIPSON, *J. Biol. Chem.*, 115 (1936) 731.
[6] B. AXELROD, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. I, Academic Press, Inc., New York, New York, 1955, p. 363.).
[7] A. C. BRATTON AND E. K. MARSHALL, *J. Biol. Chem.*, 128 (1939) 537.
[8] K. K. KOESSLER AND M. T. HANKE, *J. Biol. Chem.*, 39 (1919) 497.
[9] Z. DISCHE, in E. CHARGAFF AND J. N. DAVIDSON, *The Nucleic Acids*, Vol. I, Academic Press, Inc., New York, New York, 1955, p. 301.
[10] J. R. DYER, in D. GLICK, *Methods of Biochemical Analysis*, Vol. III, Interscience Publishers, Inc., New York, New York, 1956, p. 111.
[11] I. SMITH, *Chromatographic Techniques*, William Heineman Medical Books, Ltd., London, 1958, p. 127.
[12] H. T. GORDON, W. THORNBURG AND L. N. WERUM, *Anal. Chem*,. 28 (1956) 849.
[13] S. M. PARTRIDGE, *Nature*, 164 (1949) 443.
[14] R. KLEVSTRAND AND A. NORDAL, *Acta. Chem. Scand.*, 4 (1950) 1320.
[15] B. A. AMES AND H. K. MITCHELL, *J. Am. Chem. Soc.*, 74 (1952) 252.
[16] R. B. HURLBERT, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. III, Academic Press, Inc., New York, New York, 1957, p. 785.
[17] B. LEVENBERG AND J. M. BUCHANAN, *J. Biol. Chem.*, 224 (1957) 1005.
[18] J. G. FLAKS, M. J. ERWIN AND J. M. BUCHANAN, *J. Biol. Chem.*, 228 (1957) 201.
[19] N. O. KAPLAN, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. III, Academic Press, Inc., New York, New York, 1955, p. 874.
[20] T. R. HARKINS AND H. FREISER, *J. Am. Chem. Soc.*, 80 (1958) 1132.
[21] A. SZENT-GYÖRGYI, in O. H. GAEBLER, *Enzymes: Units of Biological Structure and Function*, Academic Press, Inc., New York, New York, 1956, p. 393.
[22] E. SHAW, *J. Am. Chem. Soc.*, 80 (1958) 3899.
[23] M. A. STEVENS, D. I. MAGRATH, H. W. SMITH AND G. B. BROWN, *J. Am. Chem. Soc.*, 80 (1958) 2755.
[24] C. E. CARTER, *J. Biol. Chem.*, 223 (1956) 139.
[25] S. H. LOVE AND J. S. GOTS, *J. Biol. Chem.*, 212 (1955) 647.
[26] D. P. LONES, C. RAINBOW AND J. D. WOODWARD, *J. Gen. Microbiol.*, 19 (1958) 146.
[27] H. FREIDMAN AND A. G. MOAT, *Arch. Biochem. Biophys.*, 78 (1958) 146.