Carbohydrate Research, 69 (1979) 89–95
© Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

ACID-CATALYZED AND ALMOND β-D-GLUCOSIDASE-CATALYZED HYDROLYSIS OF PURIN-6-YL 2-DEOXY-1-THIO-β-D-*arabino*-HEXOPYRANOSIDE

PAUL A. LARTEY AND LEO FEDOR*

Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14260 (U.S.A.)

(Received January 23rd, 1978; accepted for publication, April 18th, 1978)

ABSTRACT

Hydrolysis of purin-6-yl 2-deoxy-1-thio-β-D-*arabino*-hexopyranoside (**2**) to 6-mercaptopurine and 2-deoxy-D-glucose is catalyzed by hydronium ion and almond β-D-glucosidase. The dependence of rate on acidity in water and deuterium oxide indicates that **2** and its conjugate acid undergo hydrolysis *via* a mechanism that involves a partially rate-limiting proton transfer. Although **2** is $\sim 10^3$ more reactive than 6-purinyl β-D-glucothiopyranoside (**1**) in dilute aqueous acid, **1** is a better substrate for almond β-D-glucosidase.

INTRODUCTION

The hydrolysis of purin-6-yl 1-thio-β-D-glucopyranoside (**1**) to 6-mercaptopurine and D-glucose is catalyzed by hydronium ion, almond β-D-glucosidase, and hog liver thioglycosidase[1,2]. The dependence of rate on acidity in water and deuterium oxide indicates that both neutral and acid forms of **1** undergo hydrolysis *via* the A-1 mechanism (oxocarbonium ion formation is rate-limiting). We suggested that the 10^6-fold greater reactivity of **1** than that of phenyl 1-thio-β-D-glucopyranoside may be due in part to departure of the aglycon group as the thermodynamically more stable thioamide rather than the thioimidate, and that the proton source for N-1 could be HO-2. For β-D-glucosidase, it has been reported that 2-*O*-methyl-D-glucosides are not appreciably hydrolyzed by the enzyme[3–5], which suggests that HO-2 may play a role in the enzymic reaction. To test the possible involvement of HO-2 in acid-catalyzed and almond β-D-glucosidase-catalyzed hydrolysis of purinyl thio-D-glucosides, we synthesized purin-6-yl 2-deoxy-1-thio-β-D-*arabino*-hexopyranoside (**2**), and we report here the results of its hydrolysis in dilute acid solution and in acetate buffer containing almond β-D-glucosidase. We also prepared the known pyrid-2-yl 1-thio-β-D-glucopyranoside (**4**) so as to compare its hydrolytic lability with that of **1** and **2**.

*To whom correspondence should be addressed.

EXPERIMENTAL

General methods. — N.m.r. spectra were recorded with a Varian A-60 spectrometer with tetramethylsilane as the internal standard, and u.v. and i.r. spectra with Beckman DB-G and IR-8 spectrophotometers. The pH values were measured with a Radiometer PHM 22 instrument equipped with a PHA 630 P scale expander and a GK 2021 B electrode. Kinetics were determined on a Gilford 2400 spectrophotometer equipped with thermospacers through which water was circulated from a Tamson T-9 constant-temperature bath. Calculations were performed on a Hewlett–Packard HP 25, 9820A, or 9100A calculator programmed for linear-regression analysis. A CDC computer and the NLIN-2 program of the SUNY Computer Center Library were used for nonlinear-regression analysis.

Reagents and compounds. — Certified ACS-grade inorganic salts and organic solvents, redistilled and dried where necessary, were purchased from Fisher Scientific Co., Pittsburgh, PA 15219. Doubly glass-distilled water was used in the kinetics experiments. Deuterium oxide and deuterium chloride were purchased from Diaprep, Inc. 2-Deoxy-D-*arabino*-hexose was purchased from Calbiochem, San Diego, CA 92112, and 6-mercaptopurine and 2-pyridinethione were purchased from Aldrich Chemical Co., Inc., Milwaukee, WI 53233. Almond β-D-glucosidase (EC 3.2.1.21) was purchased from Worthington Biochemical Corp., Freehold, NJ 07728. Purin-6-yl 1-thio-β-D-glucopyranoside was available from a previous study and pyrid-2-yl 1-thio-β-D-glucopyranoside was prepared by the method of Wagner and Pischel[6], m.p. 97–100° (lit.[6] m.p. 93–101°), $[\alpha]_D^{23}$ −55.1° (*c* 2.5, *N*,*N*-dimethylformamide) {lit[6] $[\alpha]_D^{23}$ −56.4° (*c* 2.5, *N*,*N*-dimethylformamide)}.

2-Deoxy-3,4,6-tri-O-(p-*nitrobenzoyl*)-α-D-arabino-*hexopyranosyl chloride*[7] (**5**). — 2-Deoxy-1,3,4,6-tetra-*O*-*p*-nitrobenzoyl-D-*arabino*-hexose[8] (20 g, 30 mmol) was added to anhydrous dichloromethane (200 ml) saturated with hydrogen chloride. The mixture was stirred at −10° (ice-salt) for 2 h after which ~1 equiv. of *p*-nitrobenzoic acid was filtered off. The volume of filtrate was reduced to ~50 ml, ~50 ml of anhydrous ether was added, and the mixture was kept for 15 h at 5° to give a white, crystalline product (81%), m.p. 152°, $[\alpha]_D^{26}$ + 67.2° (*c* 1, dichloromethane); n.m.r. (Me_2SO-d_6): δ 2.85 (m, 2 H, H-2), 4–4.3 (m, 3 H, H-5, H-6), 4.9–5.3 (m, 2 H, H-3, H-4), 7.3–7.8 (m, 12 H, arom), and 5.4 (1 H, $J_{1,2}$ 5.5 Hz, α-D anomer).

Purin-6-yl 2-deoxy-3,4,6-tri-O-(p-*nitrobenzoyl*)-*1-thio*-β-D-arabino-*hexopyranoside* (**3**). — To a solution of sodium (0.5 g, 20 mmol) in dry methanol (150 ml) was added 6-mercaptopurine monohydrate (3.56 g, 20 mmol) with stirring. Sodium 6-mercaptopurine precipitated from the initially clear solution over 5 min. The precipitate was filtered off, washed with cold, dry methanol (3 × 10 ml), and dried. The dry salt was suspended in 1,2-dimethoxyethane (200 ml), and **5** (10 g, 20 mmol) was added with stirring under dry nitrogen. Sodium chloride was filtered off from the mixture after 15 h, the solvent was removed in a rotary evaporator, and the residue was applied to a dry, neutral alumina column. The product was separated from the reaction mixture with 19:1 (v/v) chloroform–methanol to give 50% of white crystals (from hexane),

m.p. 123°, $[\alpha]_D^{26}$ −15° (*c* 2.5, chloroform); one spot on t.l.c. (alumina-coated microscope slides, chloroform); $\lambda_{max}^{CHCl_3}$ 295 (log ε 3.45); n.m.r. ($CDCl_3$): δ 2.8 (m, 2 H, H-2), 4–4.3 (m, 3 H, H-5, H-6), 5–5.4 (m, 2 H, H-3, H-4), 5.9 (2 d, 1 H, $J_{ax,eq}$ 2.5 Hz, $J_{ax,ax}$ 8.5 Hz, H-1, β anomer), and 7.4–7.85 (m, 3 H, purine, 12 H, arom).

Anal. Calc. for $C_{32}H_{23}N_7O_{13}S \cdot 2H_2O$: C, 49.16; H, 3.49; N, 12.55. Found: C, 49.38; H, 3.56; N, 12.63.

*Purin-6-yl 2-deoxy-1-thio-β-*D-arabino-*hexopyranoside* (**2**). — Compound **4** (5 g, 8.5 mmol) was added to dry methanol saturated with ammonia (100 ml) and stirred for 15 h at 5°. The resulting solution was concentrated to 25 ml in the rotary evaporator and transferred to a nitrogen bag. Addition of excess peroxide-free, dry ether[9] to the concentrate precipitated **2**. The solvent was decanted, and the residue redissolved in dry methanol and reprecipitated with ether[9]. This process was repeated 6 times, when the compound gave one spot on t.l.c. (alumina-coated microscope slides, chloroform-methanol), white, amorphous solid (36% yield), very hygroscopic, m.p. 94° (dec.), $[\alpha]_D^{26}$ −45.3° (*c* 1.5, methanol); λ_{max}^{MeOH} 285 (log ε 3.97); n.m.r. (Me_2SO): δ 2.79 (m, 2 H, H-2), 3.9–4.4 (m, 5 H, H-3–H-6), 5.2 (2 d, 1 H, $J_{ax,eq}$ 2.5 Hz, $J_{ax,ax}$ 8.3 Hz, H-1), and 7.6–8 (3 s, 3 H, purine).

Anal. Calc. for $C_{11}H_{14}N_4O_4S \cdot 0.5H_2O$: C, 42.98; H, 4.93. Found: C, 43.20; H, 5.03.

The anomeric configurations of the 2-deoxy-D-*arabino*-hexosides were based on the quantitative conversion of **2** into hydrolysis products by β-D-glucosidase (see below) and on the H-1 signal in the n.m.r. spectra[10]. In this study, the H-1 signal for α-D anomers occurred as a broad singlet ($w_{0.5}$ ~6 Hz) in the region δ 4.5–5.5, a coupling constant *J* ~3 Hz being predicted. For β-D anomers $J_{ax,eq}$ was ~2.5 Hz and $J_{ax,ax}$ ~9 Hz; for the pair of doublets, $J_{ax,eq}$ ~3 Hz and $J_{ax,ax}$ ~10 Hz being predicted. The assignments of anomeric protons for compounds of this study followed closely those for 3-amino-2,3,6-trideoxy-L-*arabino*-hexopyranosides[11]. The exclusive formation of the α-D anomer in **5** is in contrast to the predominant formation of the β-D anomer in 1-*S*-acetyl-2-deoxy-3,4,6-tri-*O*-(*p*-nitrobenzoyl)-1-thio-β-D-*arabino*-hexopyranose, an intermediate that we synthesized but were unable to convert into **2**. This may be explained in terms of the anomeric effect[12], which may be intensified in 2-deoxyglycosides[13].

Kinetics and product analysis. — Acid-catalyzed reactions were effected under pseudo-first-order conditions at 30° in dilute hydrochloric acid, μ 0.1M (KCl) as previously described[2], and were followed by monitoring the release of 6-mercaptopurine at 325 nm. Pseudo-first-order rate constants were obtained by multiplying the slopes of plots of log $(A_\infty - A_0)/(A_\infty - A_t)$ *vs.* time by 2.303. Such plots were linear to at least three half-times. For reactions effected in D_2O, the pD was calculated by adding 0.4 to the pH meter reading[14]. For almond β-D-glucosidase-catalyzed reactions, the enzyme concentration was 0.01 mg/ml. These reactions were effected at 37° in 0.1M potassium acetate buffer, pH 5.0, and the total concentration of protein was adjusted to 0.1 mg/ml with bovine serum albumin. Reactions were started by addition of μl aliquots of **1** in *N*,*N*-dimethylformamide and **2** and **4** in

water to 3-ml cuvettes containing the enzyme. Reactions of **1** and **2** were followed by monitoring the release of 6-mercaptopurine at 325 nm. Reactions of **4** were followed by monitoring the release of 2-pyridinethione at 272 nm. The rates were calculated from the initial slopes of plots of A *vs.* time: initial velocity = slope/εt. In acetate buffer, pH 5.0, log ε = 4.25 for 6-mercaptopurine and log ε = 3.85 for 2-pyridinethione. The spectra of completed reactions of **2** resembled those of 6-mercaptopurine–2-deoxy-D-*arabino*-hexose; the spectra of completed reactions of **4** resembled those of 2-thiopyridone–2-deoxy-D-*arabino*-hexose. The conversion of **2** to products in 0.1M hydrochloric acid and in the buffered enzyme solutions was quantitative, based on the ratio (observed/actual) of molar absorptivity constants, which were 0.99 and 0.99, respectively. Similarly, the enzyme-catalyzed hydrolysis of **4** was quantitative.

RESULTS

Equation *1* describes the dependence of $k_{obs.}$, the pseudo-first-order rate

$$k_{obs.} = [a \cdot a_H(\text{or } a_D) + b \cdot a_H^2(\text{or } a_D^2)]/[c + a_H(\text{or } a_D)] \qquad (1)$$

constant, on a_H (or a_D), the activity of hydrogen (deuterium) ion for hydrolysis of **2** in dilute acid solution. The values of the constants a, b, and c in water (deuterium oxide) in the a_H (or a_D) range 0.002–0.054 for nine concentrations of acid for each solvent are: 1.96 (0.98) min^{-1}, 57(49)M^{-1} min^{-1}, and 5.33 × 10^{-3} (4.37 × 10^{-3})M, respectively*. For hydrolysis of **2** in 0.02–0.1M potassium acetate–acetic acid buffer and 5 concentrations of buffer, pH 4.3, $k_{obs.}$ = (8.93 ± 0.09) × 10^{-3} min^{-1} and the rate constants are independent of the total buffer concentration. Hydrolysis under similar conditions, but at pH 5.03, showed a linear increase of $k_{obs.}$ with an increase of the total buffer concentration, and the equation of the line is $k_{obs.}$ = 6 × 10^{-3} M^{-1} min^{-1} [total buffer] + 1.76 × 10^{-3} min^{-1}.

For the β-D-glucosidase-catalyzed reactions of **1**, **2**, and **4**, initial velocities were measured under steady state conditions. For **2** only, spontaneous hydrolysis in pH 5 acetate buffer was appreciable and initial velocities were corrected by sub-

TABLE I

MICHAELIS PARAMETERS FOR β-D-GLUCOSIDASE-CATALYZED HYDROLYSIS OF **1**, **2**, AND **4**[a]

Substrate	$K_M \times 10^3$ (M)	$V_{max} \times 10^4$ (M) min^{-1}	$V_{max} \times 10^3/K_M$ min^{-1}
1	12	7.6	64
2	1.1	0.08	7.4
4	1.1	0.41	37

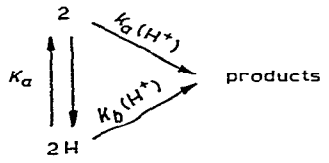
[a]Concentration range: **1**, 0.15–1.05mM; **2**, 0.1–1.12mM; **4**, 0.16–0.62mM. Temp. 37°; pH 5; μ 0.1M (KCl).

*These constants are those for which the sums of the squares of the differences between observed and calculated rate constants were minimized.

tracting the calculated rates (rate = $k_{obs.}$ [**2**]), where $k_{obs.}$ is for hydrolysis of **2** in 0.1M buffer at pH 5, from the measured enzyme rates. For **1**, **2**, and **4**, Michaelis parameters were computed in the usual way from double-reciprocal plots of $1/v_i$ *vs.* $1/[S]$, and they are provided in Table I.

DISCUSSION

Acid-catalyzed reaction. — The rate law of equation (*1*) for hydrolysis of **2** in dilute acid suggests that both **2** and its conjugate acid **2H** undergo acid-catalyzed hydrolysis according[2] to Scheme 1. Equation (*2*), derived for Scheme 1, has the form of equation (*1*). Comparison of the constants $k_a = 368\text{M}^{-1}\ \text{min}^{-1}$ and

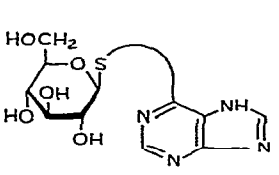


Scheme 1

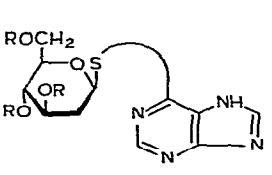
$$k_{obs.} = (k_a K_a a_H + k_b a_H^2)/(K_a + a_H) \qquad (2)$$

$k_b = 57\text{M}^{-1}\ \text{min}^{-1}$ of **2** with those[2] of **1** shows that the 2-deoxy-*arabino*-hexoside is hydrolyzed $\sim 10^3$ times faster. For those cases studied, 2-*arabino*-hexosides are more reactive than the parent glucosides by $\sim 10^3$, and this result has been attributed to electronic effects on oxocarbonium ion stability[15]. Since the difference in reactivity between **1** and **2** agrees with experimental evidence with glycosides and 2-deoxy-glycosides, assuming common elements of mechanism, we conclude that the abnormally high reactivity of **1** and **2** is likely not due to participation in the hydrolysis reaction by HO-2, but rather is due to the thiopurine group.

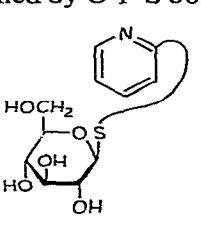
From the data just reported, the kinetic isotope effects of the deuterium solvent, $k_a(H_2O)/k_a(D_2O)$ 2.5 and $k_b(H_2O)/k_b(D_2O)$ 1.3, may be calculated. These values are in contrast to those observed for **1** (0.4 and 0.5 for k_a and k_b, respectively[2]) and for methyl 2-deoxy-α-D-*arabino*-hexopyranoside[15-17] [$k(H_2O)/k(D_2O)$ 0.4]. Whereas for the latter compound, **1** and **1H**, the isotope effects support the A-1 mechanism, which involves a fast proton transfer to the sulfur atom, followed by a rate-limiting C-1–S bond-cleavage to give an oxocarbonium ion and the aglycon, for **2** and **2H** the isotope effects support an ASE-2 type mechanism, which involves a rate-limiting proton transfer to the sulfur atom accompanied by C-1–S bond cleavage[18,19].



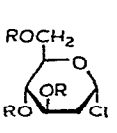
1



2 R = H
3 R = $COC_6H_4NO_2(p)$



4



5 R = $COC_6H_4NO_2(p)$

Anderson and Capon[18] and Fife[19] have called attention to the possibility that a spectrum of transition states exist for acetals and thioacetals that undergo acid-catalyzed hydrolysis, and that the transition states differ in the extent of proton transfer and C–O bond-cleavage. For general acid catalysis to occur, one or a combination of two features of A-1 reactions must be altered: (a) the basicity of the acetal oxygen (thioacetal sulfur) atom must be reduced, and (b) C–O (C–S) bond-breaking (oxocarbonium-ion formation) must be enhanced. With respect to **2**, both of these criteria are met, assuming that the proton transfer is to the sulfur atom[20]. Concerning the second feature, 6-mercaptopurine appears to be a superlative* leaving-group[2], and absence of HO-2 in **2** favors oxocarbonium-ion formation, on electronic and steric grounds[23], in contrast to **1**. Based on these theoretical considerations and the kinetic isotope effect of the deuterium solvent, hydrolysis of **2** appears to represent at least a borderline case of the ASE-2 mechanism in thioacetal hydrolysis. The results of the acetate–acetic acid buffer experiments were not definite, and, because of the possible ambiguities in interpretation of "buffer catalysis" experiments for acetal and ortho ester hydrolysis[24], we did not pursue these experiments further.

β-D-Glucosidase-catalyzed reaction. — It is generally believed that glycosidases catalyze hydrolysis of glycosides *via* oxocarbonium ions or glycosyl–enzyme intermediates. Burst kinetics have been observed for hydrolysis of *p*-nitrophenyl β-D-glucopyranoside catalyzed by β-D-glucosidase in aqueous dimethyl sulfoxide at low temperatures[25]. This, coupled with studies of secondary deuterium isotope effects[26], suggests that almond β-D-glucosidase operates *via* a glucosyl–enzyme intermediate**. For such a mechanism, it can be shown that $V_{max}/K_M = k_2E_t/K_s$ where k_2 is the rate constant for glucosyl–enzyme complex formation and K_s is the dissociation constant for the Michaelis complex. Based on this ratio, the order of substrate reactivity is **1** > **4** > **2** (Table I), and **2** is indeed a substrate for the enzyme, albeit a poorer one than **1** by a factor of ~1/10. It may be concluded that HO-2 is not a substrate requirement for β-D-glucosidase activity, and thus it does not play an obligatory catalytic role in the enzyme mechanism. This result lends support to the glycosyl–enzyme mechanism for almond β-D-glucosidase. Failure of 2-*O*-methylglucosides and **2** to serve as (good) substrates for the enzyme suggests the presence of a binding point for HO-2 that helps provide an optimum orientation of the substrate for most efficient hydrolysis. One could speculate that the enzyme accepts a hydrogen bond from the substrates, or that little bulk tolerance exists at C-2, even for a methoxyl group. Compounds **1** and **4** appear to have comparable reactivities in dilute acid[2,28], and they are less reactive than **2**. However, **1** and **4** are better enzyme substrates than **2**, which could be anticipated from the known specificity that the enzyme has for β-D-

*At pH 6.8, tetrahydro-2-(6-purinylthio)pyran is hydrolyzed with a pseudo-first-order rate constant of ~0.2 min^{-1} at 30°. This may be compared with k_{obs}. 0.0014 min^{-1} for the hydrolysis[21,22] of tetrahydro-2-(*p*-nitrophenoxy)pyran, at pH 7, in 50% aqueous 1,4-dioxane at 50°.

**A stable enzyme–D-glucose intermediate was reported for the short-term reaction between methyl α-D-glucosidase and methyl α-D-[^{14}C]glucopyranoside[27].

glucopyranoses; likely, **1** and **4** are more productively bound and oriented in the active site, and this more than compensates for the differences in inherent reactivity between **1**, **2**, and **4**.

ACKNOWLEDGMENT

This work was supported, in part, by grant CA21755 from the National Cancer Institute.

REFERENCES

1 I. GOODMAN, L. SALCE, AND G. H. HITCHINGS, *J. Med. Chem.*, 11 (1968) 516–521.
2 L. FEDOR AND B. S. R. MURTY, *J. Am. Chem. Soc.*, 95 (1973) 8410–8413.
3 H. BAUMANN AND W. PIGMAN, in W. PIGMAN (Ed.), *The Carbohydrates*, Academic Press, New York, 1957, pp. 562–601.
4 K. NISIZAWA AND Y. HASHIMOTO, in W. PIGMAN AND D. HORTON (Eds.), *The Carbohydrates*, 2nd edn., Vol. IIA, Academic Press, New York, 1970, pp. 241–300.
5 B. HELFERICH AND J. GÖRDELER, *Ber. Verh. Saechs. Akad. Wiss. Leipzig Math. Naturwiss. Kl.*, 92 (1940) 75–106.
6 G. WAGNER AND H. PISCHEL, *Arch. Pharm. (Weinheim, Ger.)*, 296 (1963) 576–590.
7 W. W. ZORBACH AND T. A. PAYNE, JR., *J. Am. Chem. Soc.*, 80 (1958) 5564–5568.
8 W. W. ZORBACH AND G. PIETSCH, *Justus Liebigs Ann. Chem.*, 655 (1962) 26–35.
9 J. A. RIDDICK AND W. B. BUNGER, in A. WEISSBERGER (Ed.), *Technique of Chemistry, Organic Solvents*, Vol. II, Wiley–Interscience, New York, 1970, pp. 690–693.
10 R. U. LEMIEUX AND J. D. STEVENS, *Can. J. Chem.*, 44 (1966) 249–262.
11 F. ARCAMONE, S. PENCO, A. VIGEVANI, S. RADAELLI, G. FRANCHI, A. DIMARCO, A. M. CASAZZA, T. DASDIA, F. FORMELLI, A. NECCO, AND C. SORANZO, *J. Med. Chem.*, 18 (1975) 703–707.
12 J. T. EDWARD, P. F. MORAND, AND I. PUSKAS, *Can. J. Chem.*, 39 (1961) 2069–2085.
13 W. W. ZORBACH AND K. V. BHAT, *Adv. Carbohydr. Chem.*, 21 (1966) 273–321.
14 T. H. FIFE AND T. C. BRUICE, *J. Phys. Chem.*, 65 (1961) 1079.
15 B. CAPON, *Chem. Rev.*, 69 (1969) 407–498.
16 C. ARMOUR, C. A. BUNTON, S. PATAI, L. H. SELMAN, AND C. A. VERNON, *J. Chem. Soc.*, (1961) 412–416.
17 W. G. OVEREND, C. W. REES, AND J. S. SEQUEIRA, *J. Chem. Soc.*, (1962) 3429–3440.
18 E. ANDERSON AND B. CAPON, *J. Chem. Soc. B*, (1969) 1033–1037.
19 T. H. FIFE, *Acc. Chem. Res.*, 5 (1972) 264–272.
20 C. BAMFORD, B. CAPON, AND W. G. OVEREND, *J. Chem. Soc.*, (1962) 5138–5141.
21 L. FEDOR, unpublished result.
22 T. H. FIFE AND L. K. JAO, *J. Am. Chem. Soc.*, 90 (1968) 4081–4085.
23 W. G. OVEREND, C. W. REES, AND J. S. SEQUEIRA, *J. Chem. Soc.*, (1962) 3431–3440.
24 P. SALOMAA, A. KANKAANPERÄ, AND M. LAHTI, *J. Am. Chem. Soc.*, 93 (1971) 2084–2085.
25 A. L. FINK AND N. E. GOOD, *Biochem. Biophys. Res. Commun.*, 58 (1974) 126–131.
26 F. H. DAHLQUIST, T. RAND-MEIR, AND M. A. RAFTERY, *Biochemistry*, 8 (1969) 4212–4221.
27 H.-Y. L. LAI, L. G. BUTLER, AND B. AXELROD, *Biochem. Biophys. Res. Commun.*, 60 (1974) 635–639.
28 G. WAGNER AND R. METZNER, *Naturwissenschaften*, 52 (1965) 83–84.