Neoprontosil Binding to Carbonic Anhydrase. Resonance Raman and Other Studies on the Ionization Behavior of the Sulfonamide†

P. R. Carey* and R. W. King

ABSTRACT: Alkalimetric, spectrophotometric, NMR, and resonance Raman titrations are reported for the sulfonamide Neoprontosil in aqueous solution. An assignment of the magnetic resonance peaks for each of the Neoprontosil protons has been made. Neoprontosil is shown to have two "coupled" ionizable groups, -OH and $-SO_2NH_2$, in the pH range 10.5–11.5. The close proximity of the microscopic p*K*s for these two groups precludes spectroscopic characterization of the separate $-SO_2NH_2$, $-O^-$ or $-SO_2NH^-$, -OH species. For this reason, no conclusion can be drawn on the ionization state of the drug when bound to carbonic anhydrase. The resonance Raman spectrum of Neoprontosil bound to human carbonic anhydrase B at pH 9.5 shows a shift in the intense -N=N- stretching mode from 1414 (free) to 1407 cm^{-1} (bound), suggesting that a slight conformational change about the -N=N- single bond linkages occurs upon binding.

The zinc metalloprotein carbonic anhydrase (EC 4.2.1.1) catalyzes the reversible hydration of CO_2. The catalytic activity of the enzyme is strongly inhibited by aromatic sulfonamides and, because of their effect on various physiological reactions ultimately involving HCO_3^-, sulfonamides have proven to be of pharmacological value (Goth, 1972). Sulfonamides, moreover, have been extremely useful in work on the physicochemistry and mechanism of action of carbonic anhydrase (Coleman, 1973; King & Burgen, 1976). There is much spectroscopic and crystallographic evidence (Lindskog & Thorslund, 1968; Lindskog et al., 1971) that the sulfonamide enters into the coordination sphere of the protein's zinc atom to form an inner sphere complex. Evidence from the ultraviolet difference spectrum of several aromatic sulfonamides (King & Burgen, 1970) and the fluorescence spectrum of bound dansylamide (Chen & Kernohan, 1967) led to the conclusion that the sulfonamide in its bound form is present as the anion, $-SO_2NH^-$.

Resonance Raman (RR) spectroscopy provides a sensitive probe for the structure of binding sites in proteins and for obtaining detailed information on changes occurring in chromophoric ligands upon binding (Carey, 1978; Carey & Schneider, 1978). Studies by this technique of three sulfonamidoazobenzene derivatives bound to a variety of carbonic anhydrases provided direct evidence for $-SO_2NH^-$ in the complex (Kumar et al., 1974, 1976), thus confirming the earlier spectroscopic studies. Furthermore, the RR work was able to eliminate hydrophobic bonding and twisting in the Ph—N=N—Ph bonds as sources of the observed spectral changes. A recent RR study by Petersen et al. (1977) creates an anomaly, however, in that these authors claim to have evidence for the sulfonamide 4′-sulfamylphenyl-2-azo-7-acetamido-1-hydroxynaphthalene-3,6-disulfonate (Neoprontosil) binding in the $-SO_2NH_2$ form. The object of the present paper is to explain this anomaly. Further RR work has been undertaken on Neoprontosil in its free and bound forms together with detailed NMR and alkalimetric and spectrophotometric titrations of the free ligand. It is shown that Neoprontosil has two ionizable groups, -OH and $-SO_2NH_2$, whose p*K*s are sufficiently close that the distinction between microscopic and macroscopic dissociation constants discussed by Edsall et al. (1958) must be employed. In practice this means that the spectroscopic properties of the $-SO_2NH_2$, O^- and $-SO_2NH^-$, OH forms cannot be separately charcterized by varying pH. Consequently, no definitive conclusion can be drawn about the ionization state of Neoprontosil bound to carbonic anhydrase.

Experimental Section

Neoprontosil (azosulfamide) was purchased from Sigma Ltd. Purity was tested by thin-layer chromatography in eight different solvent systems, and only very minor impurities were revealed.

^1H NMR spectra were recorded on a Bruker WH-270 NMR spectrometer by using a 0.5 M solution in D_2O and collecting 50–100 transients. Natural abundance ^{13}C NMR spectra and off-resonance heteronuclear decoupling experiments were obtained by using a Varian Associates XL-100 spectrometer. In this case, 5000 transients were collected. All experiments were performed at 25 °C.

Ultraviolet and visible spectra were recorded on a Cary 118 spectrophotometer by using 1-cm path-length cells.

Alkalimetric titration experiments were carried out at 25 °C by using 5 mL of 17.5 mM Neoprontosil solution constantly gassed with N_2 and stirred. A Radiometer PHM 26 pH meter fitted with a Russell pH Ltd. CMAT 7/5 electrode was used for pH measurement. Standard 1.0 M KOH solution was added by an "Agla" micrometer syringe. As far as possible, the whole of the titration curve, corrected for a blank titration, was recorded.

Resonance Raman spectra were recorded as described previously (Kumar et al., 1976). Measurements with 406.7- and 413.1-nm excitation used a Coherent Radiation 3000K krypton laser and a Spex 1/2m spectrometer equipped with dc detection. Each data point shown in Figure 3 is the mean of at least three measurements.

The concentration and activity of carbonic anhydrase were measured as described elsewhere (Kumar et al., 1976). The absorption spectrum of Neoprontosil bound to the human carbonic anhydrase B used in our studies corresponded closely to that reported by Petersen et al. (1977) for the drug bound to bovine carbonic anhydrase.

Results and Discussion

Ionization Behavior of Neoprontosil. Figure 1 contains the results of spectrophotometric and alkalimetric titrations of Neoprontosil between pH 9 and 13. The alkalimetric titration

† From the Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6 (P.R.C.), and the National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW7 1AA, United Kingdom (R.W.K.). *Received December 1, 1978.* This is NRCC No. 17500.

0006-2960/79/0418-2834$01.00/0

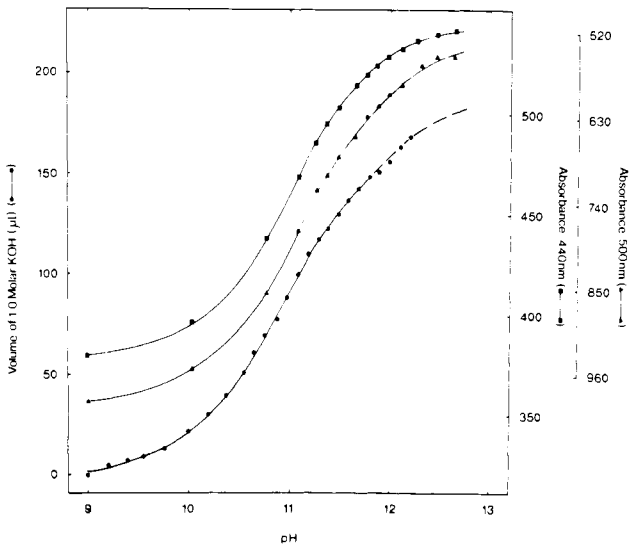


FIGURE 1: Alkalimetric and spectrophotometric titration of Neoprontosil. The alkalimetric titration was of 5 mL of 17.5 mM Neoprontosil solution and was corrected for a blank titration. Spectrophotometric titration was carried out by using 1-cm path-length cells. Scale of absorbance is for a 40 mM solution.

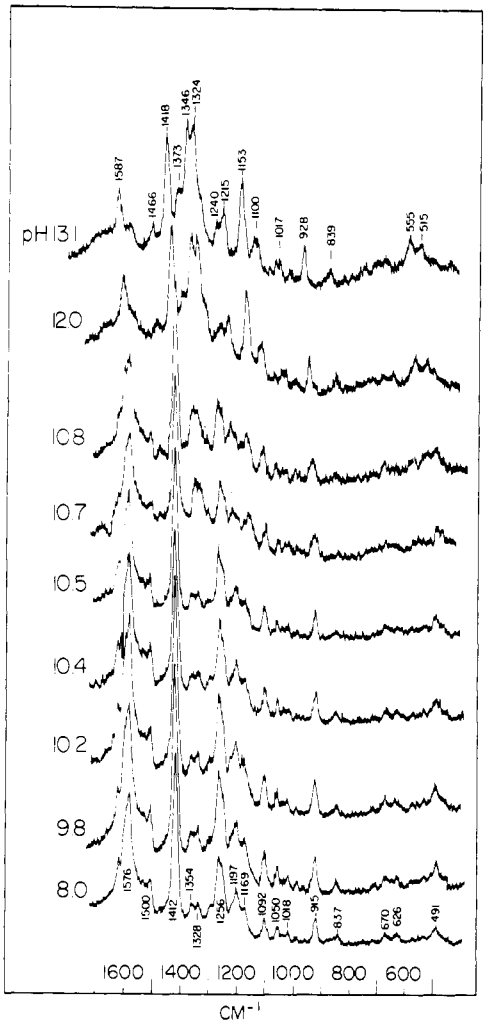


FIGURE 2: Resonance Raman spectra of Neoprontosil (2.5×10^{-4} M) at various pHs; 457.9-nm excitation, 50 mW; 9-cm^{-1} spectral slit.

shows that, between pH 9 and 12, 1.8 equiv of KOH was neutralized by ionization of the compound, and, this, taken with the shape of the spectrophotometric titration curves, clearly indicates that two groups are ionizing in this range.

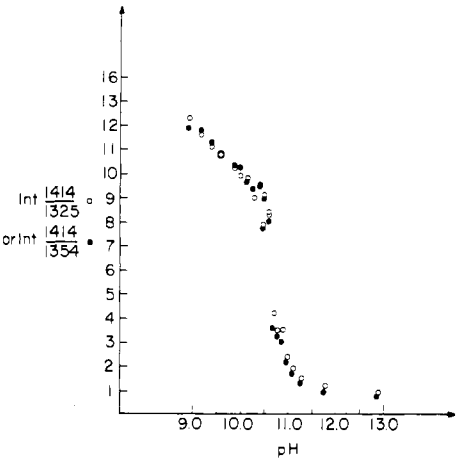


FIGURE 3: Resonance Raman titration of Neoprontosil (7.5×10^{-5} M in H_2O). Laser line, 457.9 nm, ~85 mW; 9-cm^{-1} spectral slit.

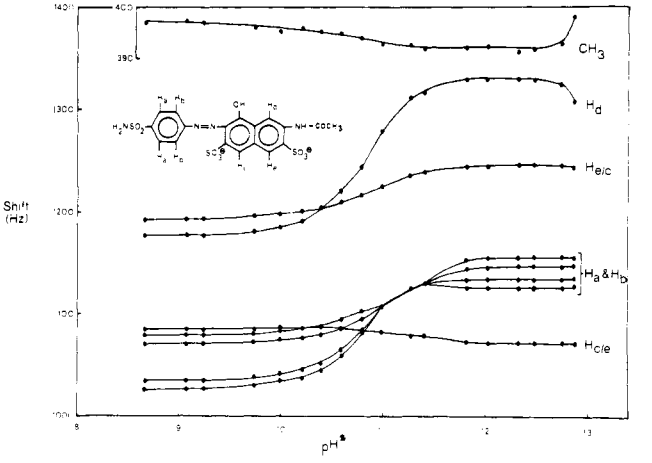


FIGURE 4: Proton magnetic resonance titration of Neoprontosil. Protons H_a–H_e are all affected by the titration of the $-SO_2NH_2$ and –OH groups.

The alkalimetric titration curve can be fitted reasonably well assuming a pair of independently titrating groups with pK values of 10.7 and 11.3. However, by using these values, it is not possible to fit either of the absorption change against pH curves.

A sample of the RR spectra recorded between pH 8.0 and 13.1 with 457.9-nm excitation is shown in Figure 2. In general accord with the alkalimetric data, a plot of the relative RR intensities of the 1414 and 1325 or 1354 cm^{-1} features (Figure 3) reveals that these spectra are extremely sensitive to changes in pH near pH 10.6. The changes in the limbs of the RR titration curve are sufficiently small that the RR spectra in the region of pH 10.6 could all be reproduced to within experimental error, by a weighted superposition of spectra taken at pH 9.0 and 12.0.

Neoprontosil (Figure 4) contains three ionizable groups. These are the $-SO_2NH_2$ group, the –OH group, and the $-NH-COCH_3$ group. The pK of the $-SO_2NH_2$ group in many other sulfonamides ranges from 9.2 to 10.4 (King & Burgen, 1976), and even in extremely favorable cases such as pentafluorobenzenesulfonamide the pK only falls to 8.1 (Olander et al., 1973). This suggests that the orbital overlap of the ionized sulfonamide group with the π system of the rest of the molecule is somewhat restricted. The pK of α-naphthol is 9.34 at 25 °C, in itself 0.5 pH unit lower than that of phenol, while the pK of acetanilide is 13 at 25 °C (*Handbook of Chemistry and Physics*, 1968). Thus the groups ionizing in the range

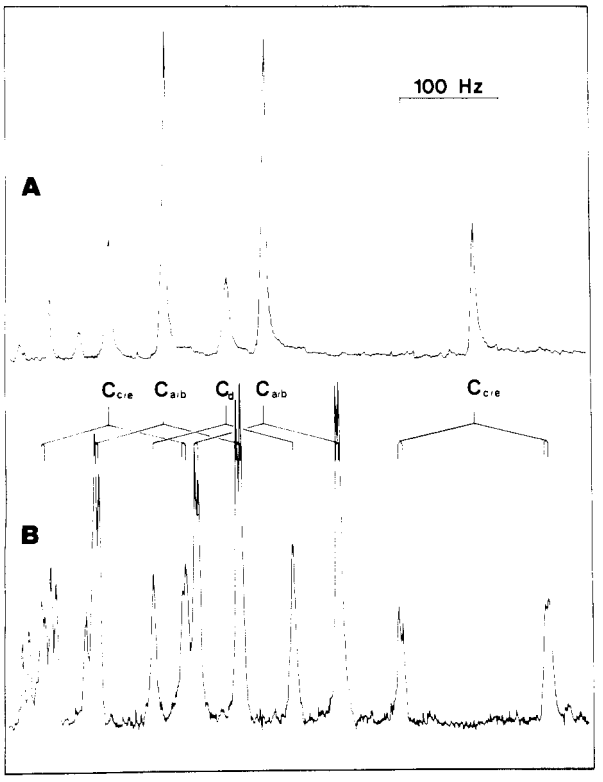


FIGURE 5: Carbon-13 magnetic resonance spectrum of Neoprontosil. (A) Full proton decoupling power gives simplified spectrum. Large pulse width allows identification of -CH carbons and of $C_{a/b}$ carbons. (B) Off-resonance proton decoupled spectrum allows identification of C_d and of $C_{c/e}$ carbons through fine-splitting of peaks. Spectra recorded at 25 °C, pH 12, saturated solution.

pH 9–12 are most likely the sulfonamide and naphtholic groups.

The titration behavior of the molecule has also been followed by proton NMR as shown in Figure 4. Here the shifts of the proton peaks have been plotted as a function of pH*, the uncorrected pH meter reading of the solution in D_2O. The proton resonances have been identified by a combination of ^1H and ^{13}C magnetic resonance spectroscopy. The C–H carbons (Figure 5A) were identified by their shorter relaxation times (higher intensities) in an experiment using a large pulse width. The benzenoid C–H carbons were identified by their higher intensities leaving three resonances representing C_c, C_d, and C_e in the naphthalene ring. C_c and C_e were identified in an experiment with no proton-decoupling power as shown in Figure 5B, where the fine splitting due to three-bond C–H couplings is visible on four of six peaks due to C_c, C_d, and C_e; hence, C_d is identified. By an off-resonance heteronuclear decoupling experiment (Birdsall et al., 1972; Freeman & Hill, 1971) H_a–H_e have been assigned as shown in Figure 4.

The ionization of the sulfonamide and naphtholic groups produced shifts in *all* of the proton resonances. We have found it impossible to fit the curves using a simple sequential ionization mechanism of the

$$HA\text{-}P\text{-}BH \overset{K_1}{\rightleftharpoons} A^-\text{-}P\text{-}BH \overset{K_2}{\rightleftharpoons} A^-\text{-}P\text{-}B^-$$

type. This, taken in conjunction with the results shown in Figure 1, suggests that the pKs of the sulfonamide and phenolic functions are sufficiently close together that the mechanism which operates is given in Scheme I. For a dibasic acid an alkalimetric titration, in which pH is determined as a function of the mean number of protons removed from HA′H by addition of hydroxyl ions, does not give the above microscopic constants k_1, k_2, k_{12}, and k_{21} but two macroscopic constants

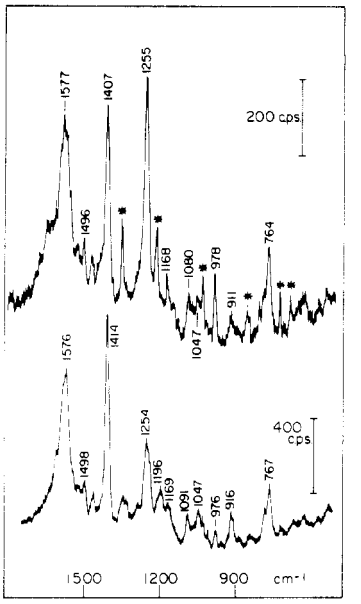
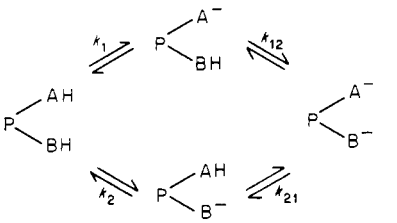


FIGURE 6: Resonance Raman spectra of (bottom) free aqueous Neoprontosil (3×10^{-5} M) and of (top) Neoprontosil (3×10^{-5} M) bound to human carbonic anhydrase B (10^{-4} M). Both spectra were taken in Tris buffer at pH 9.5. Laser line, 457.9 nm, 50 mW; 9-cm^{-1} spectral slit. Asterisks denote laser plasma lines. The 978-cm^{-1} feature in bound form may have contribution from SO_4^{2-}.

Scheme I



K_1 and K_2 (Edsall et al., 1958). Moreover, the microscopic constants are likely to be closer to each other than any macroscopic pK values measured, and, in this case where the measured macroscopic constants are only separated by 0.6 pH unit, they are likely to be very close indeed. Since the properties of both the intermediate states are unknown, it is not surprising that a fit cannot be achieved to the curves of changes in spectroscopic properties with pH.

Resonance Raman Spectrum of Neoprontosil Bound to Carbonic Anhydrase. In Figure 6 the RR spectrum of free aqueous Neoprontosil at pH 9.5 is compared with that of the ligand bound to human carbonic anhydrase B at the same pH. At the concentrations employed no features from unbound ligand appear in the spectrum of the bound form. The pH of 9.5 was chosen because, on the basis of the preceding section, there is no ambiguity as to the ionization state of the free ligand. It is in the $-SO_2NH_2$, -OH form. In contrast to the findings of Petersen et al. (1977) who stated no changes on binding in the pH range 6.0–11.2, we observe marked differences. Upon binding there is an increase in intensity of the 1255-cm^{-1} peak and crucially a 7-cm^{-1} shift to lower cm^{-1} of the 1414-cm^{-1} feature assignable to the N=N stretching vibration $\nu_{N=N}$ (Kumar & Carey, 1977). The shift in $\nu_{N=N}$ is particularly important since, while Raman intensities reflect electronic ground and excited state properties, Raman peak positions are a property solely of the electronic ground state. Thus, the shift in $\nu_{N=N}$ unambiguously reflects a change in chemistry, i.e., in conformation and/or ionization state, of the ligand upon binding. Although the absorption spectra of

Neoprontosil bound to bovine carbonic anhydrase (Petersen et al., 1977) and the human enzyme B form (this work) are identical, it is still possible that the differences observed in the RR spectra upon binding by the two groups reflect slight differences in binding sites of the two forms of carbonic anhydrase.

The determination of the ionization state of bound Neoprontosil by RR spectroscopy requires as standards the RR spectra of the four species: $-SO_2NH_2$, $-OH$; $-SO_2NH^-$, $-OH$; $-SO_2NH_2$, $-O^-$; and $-SO_2NH^-$, $-O^-$. Only then is it possible to be certain that changes observed upon binding at any pH are due to changes in the ionization of one or the other group. Moreover, changes due to alterations in Neoprontosil "backbone" conformation upon binding or perturbations due to changing dielectric must not be confused with ionization properties.

Attempts at specifically methylating the –OH group of Neoprontosil were unsuccessful. Thus, we do not have a RR spectrum of either of the $-SO_2NH_2$, $-O^-$ or $-SO_2NH^-$, $-OH$ or $-SO_2NH^-$, $-OCH_3$ forms for standards for comparison with the bound ligand at pH 9.5. On the basis of comparison with the free ligand in the $-SO_2NH^-$, $-O^-$ state (Figure 2, pH 13.1), it seems certain that Neoprontosil does not bind, at pH 9.5, in the doubly ionized form. Furthermore, as discussed below, we believe that –OH ionization is not taking place. However, the possibility of a change in conformation about the –N=N– linkage occurring upon binding clouds further interpretation. For three other sulfonamides studied previously, viz., 4-sulfonamido-4′-dimethylaminoazobenzene, 4-sulfonamido-4′-hydroxyazobenzene, and 4-sulfonamido-4′-aminoazobenzene (Kumar et al., 1974, 1976), binding to carbonic anhydrase caused no shift in $\nu_{N=N}$. For Neoprontosil the 7-cm^{-1} decrease in $\nu_{N=N}$ in the bound form may indicate a decrease in double-bond character reflecting an increase in electron conjugation through the –N=N– linkage. This is consistent with an increase in planarity in the Neoprontosil as the ligand binds to the enzyme. The bulky nature of Neoprontosil compared with the azosulfonamides studied previously may mean that Neoprontosil is forced into a more planar configuration in the active site. By analogy with the results on –C=C– frequencies in cyclic hydrocarbons (Bellamy, 1968), an alternative explanation for the decrease in $\nu_{N=N}$ is that a change occurs in one or both of the C—N=N angles. In either case, the RR data suggest that a slight conformational change in the Neoprontosil backbone occurs upon binding.

To gain possible insight into the intensity changes observed upon binding, a Raman excitation profile of the free ligand was measured. This determines relative Raman intensities as a function of laser excitation wavelength and may be considered as a fine probe of the absorption spectrum. By using Neoprontosil concentrations of 2.5×10^{-4} M at pH 9.5, it was possible to record Raman spectra with 406.7, 413.1, 457.9, 476.5, 488.0, 496.5, 501.7, and 514.5 nm laser excitation wavelengths. At the lower end of this range, spectral quality was impaired by poor signal to noise and at the higher end by a luminescent background. However, the overall spectral appearance was clearly the same at each wavelength and in particular the intensity ratio of the 1414- and 1255-cm^{-1} features did not vary by more than ±17% throughout the range 406.7–514.5 nm. This is in general accord with the findings of Petersen et al. (1977) over the more restricted range 457.9–514.5 nm. The observed 300% increase in the 1255 cm^{-1} to 1414 cm^{-1} intensity ratio upon binding (Figure 6), therefore, cannot be accounted for by a change in the position of the Raman excitation wavelength within the absorption band. Although the intensity change upon binding thus reflects a chemical perturbation in Neoprontosil, the likelihood of conformational change taking place about the –N=N– linkage and the absence of data on the singly ionized ligand prevents precise determination of the origin of this change.

There is good, but not conclusive, support for the idea that major changes in the RR and absorption spectra of Neoprontosil occurring as a function of pH result from –OH and not from $-SO_2NH_2$ ionization. As noted in the previous section, the small variation of $-SO_2NH_2$ p*K*s among many sulfonamides suggests restricted orbital overlap between the sulfonamide grouping and the rest of a π-electron chromophore. This is strongly supported by earlier RR studies in which it was shown that only minor spectral changes resulted from $-SO_2NH_2$ going to $-SO_2NH^-$ (Kumar & Carey, 1977, and references cited therein). In terms of physical–organic chemistry, this can be expressed by saying that $-SO_2NH_2$ and $-SO_2NH^-$ have very similar Hammett constants. In contrast, the –OH and $-O^-$ moieties have very different Hammett constants; they are $\sigma_{para} = 0.37$ and -1.00 for –OH and $-O^-$, respectively (Hine, 1962). Accordingly, large changes are always observed in the RR spectra of a chromophore when a conjugated –OH group ionizes (Kumar & Carey, 1977). This is why we eliminate the $-SO_2NH_2$, O^- form, which on this basis will have a RR spectrum similar to the spectrum at pH 13.1 for the free ligand (Figure 2), from the possible causes of the spectral changes occurring in Figure 6. By the same token the likely cause of the RR spectral changes occurring when Neoprontosil binds to carbonic anhydrase at pH 11.2 (Petersen et al., 1977) is $-O^-$ going to –OH. At pH 11.2, Petersen et al. (1977) claim that Neoprontosil is mainly in the $-SO_2NH^-$, OH form and that the changes in the RR spectrum upon binding to carbonic anhydrase indicate the presence of the $-SO_2NH_2$, OH form in the active site. However, on the basis of the "coupled" nature of the $-SO_2NH$ and –OH ionizations revealed by the NMR and other data presented above we believe it is not possible to identify the species at pH 11.2 as predominately $-SO_2NH^-$, OH. Substantial amounts of $-SO_2NH_2$, O^- are likely to be present also and the large change in RR spectrum upon binding reported by Petersen et al. (1977) could equally reflect the $-SO_2NH_2$, $-O^-$, or $-SO_2NH^-$, O^- forms going to $-SO_2NH_2$, –OH, or SO_2NH^-, OH.

Acknowledgments

We are grateful to P. C. Turner, P. J. Cayley, and Dr. B. Kimber for assistance with the measurement and interpretation of NMR spectra and to R. G. Carriere for measurement of the Raman spectra. We are also grateful to a referee who pointed out a second interpretation for the observed shift in $\nu_{N=N}$.

References

Bellamy, L. J. (1968) *Advances in Infrared Group Frequencies*, pp 22–26, Methuen, London.

Birdsall, B., Birdsall, N. J. M., & Feeney, J. (1972) *Chem. Commun.*, 316.

Carey, P. R. (1978) *Q. Rev. Biophys. 11*, 309.

Carey, P. R., & Schneider, H. (1978) *Acc. Chem. Res. 11*, 122.

Chen, R. F., & Kernohan, J. C. (1967) *J. Biol. Chem. 242*, 5813.

Coleman, J. E. (1973) *Inorg. Biochem. 1*, 488.

Edsall, J. T., Martin, R. B., & Hollingworth, B. R. (1958) *Proc. Natl. Acad. Sci. U.S.A. 44*, 505.

Freeman, R., & Hill, H. D. W. (1971) *J. Chem. Phys. 54*, 3367.
Goth, A. (1972) *Medical Pharmacology*, 6th ed., p 413, C. V. Mosley Co., Saint Louis, MO.
Handbook of Chemistry and Physics (1968) 48th ed., p D-91, Chemical Rubber Publishing Co., Cleveland, OH.
Hine, J. L. (1962) *Physical Organic Chemistry*, p 87, McGraw Hill, New York.
King, R. W., & Burgen, A. S. V. (1970) *Biochim. Biophys. Acta 207*, 278.
King, R. W., & Burgen, A. S. V. (1976) *Proc. R. Soc. London, Ser. B 193*, 107–125.
Kumar, K., & Carey, P. R. (1977) *Can. J. Chem. 55*, 1444.
Kumar, K., King, R. W., & Carey, P. R. (1974) *FEBS Lett. 48*, 283.
Kumar, K., King, R. W., & Carey, P. R. (1976) *Biochemistry 15*, 2195.
Lindskog, S., & Thorslund, A. (1968) *Eur. J. Biochem. 3*, 453.
Lindskog, S., Henderson, L. E., Kannan, K. K., Liljas, A., Nyman, P. O., & Strandberg, B. (1971) *Enzymes, 3rd Ed. 5*, 587.
Olander, J. Bosen, S. F., & Kaiser, E. T. (1973) *J. Am. Chem. Soc. 95*, 4473.
Petersen, R. L., Li, T.-Y., McFarland, J. T., & Watters, K. L. (1977) *Biochemistry 16*, 726.

Dialdehydes Derived from Adenine Nucleosides as Substrates and Inhibitors of Adenosine Aminohydrolase†

Alan J. Grant and Leon M. Lerner*

ABSTRACT: A series of nucleoside dialdehydes have been obtained as powders after treatment of various adenine nucleosides with paraperiodic acid. Thus, oxidation gave dialdehydes derived from adenosine (**1**), 9-α-D-mannopyranosyladenine (**2**), 9-(5-deoxy-α-D-arabinofuranosyl)adenine (**3**), 9-α-L-rhamnopyranosyladenine (**4**), 9-β-L-fucopyranosyladenine (**5**), 9-β-D-fucopyranosyladenine (**6**), 9-α-D-arabinopyranosyladenine (**7**), 9-β-D-ribopyranosyladenine (**8**), and 9-(5-deoxy-β-D-*erythro*-pent-4-enofuranosyl)adenine (**9**). Nucleoside dialdehydes **1**–**3** and **6**–**8** were weak substrates for adenosine aminohydrolase from calf intestinal mucosa. Dialdehyde **8** had the strongest affinity, but **1** had the highest V_{max}. All of the dialdehydes except **5** were inhibitors of the enzyme. The best inhibitors were **9** (K_i = 4 μM) and **4** (K_i = 28 μM), and neither were substrates. The inhibitors did not exhibit time-dependent inhibition and did not appear to form covalent bonds with the protein. The data strongly suggest that the active form of the dialdehydes is as the open-chain dihydrates. The alcohol obtained by reduction of **9** (compound **10**) was the strongest inhibitor (K_i = 0.9 μM) among the related alcohols and the nucleoside dialdehydes.

Periodate oxidation of nucleosides yields compounds referred to as nucleoside dialdehydes. It is generally recognized that these compounds are actually equilibrium mixtures of various cyclic and acyclic hemiacetals and hydrates (Guthrie, 1961; Khym & Cohn, 1960; Jones et al., 1976; Hansske & Cramer, 1977). The nomenclature and structural representation as the aldehyde form used in publication are a matter of convenience to simplify discussion, and since they react as typical aldehydes, the different structures could usually be ignored. The closest analogy to this chemistry would be the free sugars which, in solution, exist mainly as cyclic hemiacetals rather than as straight-chain aldehydes. In recent years, the nucleoside dialdehydes have been shown to bind to a number of enzymes. Ribonucleotide reductase from Ehrlich ascites tumor cells was inhibited by the periodate oxidation products of adenosine (Cory et al., 1976) and inosine (Cory & Mansell, 1975). *S*-Methylthioinosine dialdehyde inhibited enzymes as varied as thymidylate kinase and DNA polymerase (Kimball et al., 1968), bacterial RNA polymerase (Nixon et al., 1972), and pancreatic ribonuclease (Spoor et al., 1973). Inosine dialdehyde formed stable complexes with lysine, glycine, histidine, and bovine serum albumin. The latter was shown to be cross-linked by the periodate oxidation products of inosine, adenosine, cytidine, and methyl β-D-ribofuranoside (Cysyk & Adamson, 1976). This cross-linking property was consistent with the time-dependent nature of enzyme inhibition, macromolecular synthesis, and cell proliferation (Cory et al., 1976). The finding that other aldehydes have similar effects on the same systems has led some workers to believe that cross-linking, either by Schiff base or carbinolamine formation, is the major mode of interaction of nucleoside dialdehydes and proteins (Cysyk & Adamson, 1976; Cory et al., 1976; Plagemann et al., 1977). In either case the mechanism of formation of the covalent bonds requires the hydrate form of the aldehydes (Milch, 1964). It became necessary to see how changes in structure of the nucleoside dialdehydes would affect this process and to better ascertain the range of other mechanistic possibilities. We have begun by investigating the binding of a number of analogues of adenosine dialdehyde to adenosine aminohydrolase (EC 3.5.4.4), an enzyme that does not react to any great extent with small aldehydes despite the availability of lysine residues.

Experimental Section

General Materials and Methods. Adenosine, DL-glyceraldehyde, pyridoxal, and pyridoxal phosphate were purchased from Sigma Chemical Co. Adenosine was recrystallized three times from water and dried under high vacuum at 80 °C over phosphorus pentoxide. Formaldehyde (37.2% in water) was purchased from Fisher Scientific Co., and glutaraldehyde

† From the Department of Biochemistry, State University of New York, Downstate Medical Center, Brooklyn, New York 11203. *Received December 29, 1978.* This work is taken from a thesis of A.J.G. to be submitted to the School of Graduate Studies in partial fulfillment of the requirements for the Ph.D. degree. Part of this work was supported by Research Grant CA-13802 from the National Institutes of Health.

0006-2960/79/0418-2838$01.00/0