

REACTIVITY OF THE HYDROXYL RADICAL WITH AMYGDALIN IN AQUEOUS SOLUTION

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Abstract—Rate constants were determined for the reactions of hydroxyl radicals with amygdalin in aqueous solutions by means of the pulse radiolysis technique. The overall rate constant (phenyl group addition and H-atom abstraction) was $4.1 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$ at 22° . The addition rate constant was $3.7 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$. The abstraction rate constant was in the order of $4 \times 10^8 \text{ M}^{-1} \text{ sec}^{-1}$. This high reactivity is pertinent to models for protection, by amygdalin, against alloxan-induced diabetes.

It has been suggested [1-7] that the active agent in alloxan-induced diabetes is the hydroxyl radical, OH, a powerful oxidizing species [8, 9] which can be generated from alloxan as part of a redox cycle [10-12]. This suggestion was based on the observation that alloxan-induced diabetes in mice was prevented by treatment with a number of compounds, including amygdalin [1, 2], all of which are effective scavengers of OH.

Amygdalin in aqueous solution would be expected to react with hydroxyl radicals, because it contains a phenyl group to which OH may add, and a sugar structure from which OH may abstract hydrogen. It may be expected [8, 9] by analogy with a variety of aromatic compounds that the addition reaction would have a rate constant on the order of $5 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$ at 22° , and the abstraction reaction a somewhat lower rate constant.

In the foregoing context it was of interest to determine the value of the rate constants by means of the pulse radiolysis technique [13, 14]. We report these experimental results here.

EXPERIMENTAL

The apparatus in use with a Varian V-7715A electron linear accelerator has been described previously [15]. Four MeV electrons with a pulse duration of 500-1000 nsec were used for this work. The dose per pulse was about $1 \times 10^{17} \text{ eV/g}$, giving an initial OH concentration of about $10 \mu\text{M}$. The 2 cm quartz irradiation cell was constructed so it could be filled in the absence of air, and a syringe technique, described elsewhere [16, 17], was employed for filling the cell. A double pass of the analyzing light beam was utilized. All experiments were performed at 22° .

Ferrocyanide ion solutions were used as the reference standard for the competition kinetics. The optical absorption of the ferricyanide ion formed

upon irradiation has an absorption maximum at 410 nm. Accordingly, the optical density produced upon pulse irradiation was determined for $170 \mu\text{M}$ $\text{K}_4[\text{Fe}(\text{CN})_6]$ solutions at 410 nm. The optical densities of nine irradiated solutions containing both ferrocyanide and amygdalin were also measured at 410 nm. These solutions, all of which were $170 \mu\text{M}$ $\text{K}_4[\text{Fe}(\text{CN})_6]$, ranged from 160 to 870 μM amygdalin.

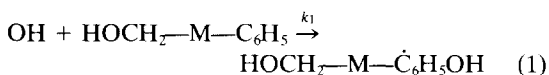
For the determination of the rate constant for the reaction of the OH radical with amygdalin to form the hydroxycyclohexadienyl product, seven solutions of amygdalin, having almost a 6-fold concentration range, were prepared. A fresh solution was used for each irradiation, and a minimum of nine determinations was done for each solution.

All solutions, prepared with triply distilled water, were bubbled with N_2O , to deaerate the solution while saturating it with N_2O , which scavenges the hydrated electron converting it to OH [13]. The amygdalin, obtained from the Sigma Chemical Co. (St. Louis, MO) was 99 per cent pure, and the $\text{K}_4[\text{Fe}(\text{CN})_6]$ was a Baker Analyzed reagent.

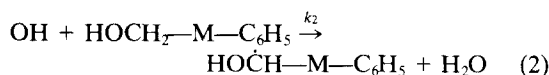
RESULTS AND DISCUSSION

The reaction of the OH radical with amygdalin involves two separate processes:

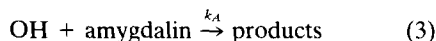
(a) An addition reaction to form the hydroxycyclohexadienyl radical product,



(b) a reaction resulting in hydrogen abstraction of any accessible hydrogen,

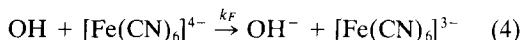


The overall reaction, with $k_A = k_1 + k_2$, is:



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By competition experiments, employing a reference standard such as ferrocyanide ion, the k_A value may be readily determined. The ferrocyanide ion competes with the amygdalin for the OH radical as shown:



The ferricyanide ion product has a characteristic absorption spectrum with a maximum at 410 nm, whereas the products of OH reaction with amygdalin do not absorb significantly at this wavelength. k_F for reaction 4 has been reported [9, 18] as $0.93 \times 10^{10} \text{ M}^{-1} \text{ sec}^{-1}$. Since the bimolecular rate constant for OH recombination, $2k = 1.2 \times 10^{10} \text{ M}^{-1} \text{ sec}^{-1}$, this reaction has negligible influence on the kinetics of OH removal at the doses and ferrocyanide ion concentration employed.

Considering the reactions of equations 3 and 4, the relative probability that the OH radical will react with the ferrocyanide ion in solutions containing both ferrocyanide ion and amygdalin is given by:

$$\text{Probability} = \frac{k_F[\text{Fe}(\text{CN})_6]^{4-}}{k_F[\text{Fe}(\text{CN})_6]^{4-} + k_A[\text{amygdalin}]} \quad (5)$$

The optical densities of irradiated ferrocyanide solutions without, D_0 , and with, D , amygdalin present were measured at 410 nm and are related as shown:

$$\frac{D_0^{410}}{D^{410}} = 1 + \frac{k_A[\text{amygdalin}]}{k_F[\text{Fe}(\text{CN})_6]^{4-}} \quad (6)$$

According to equation 6, D_0/D plotted as a function of relative solute concentration should give a straight line with a slope equal to k_A/k_F and an intercept of unity. This plot is shown in Fig. 1. Using $k_F = 0.93 \times 10^{10} \text{ M}^{-1} \text{ sec}^{-1}$, [18], k_A was found to be $(4.1 \pm 0.3) \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$.

This ultraviolet absorption band formed in irradiated amygdalin solutions is shown in Fig. 2. The

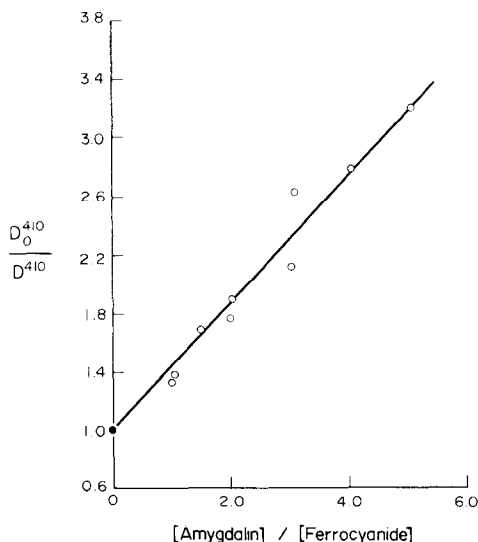


Fig. 1. Competition of ferrocyanide ion and amygdalin for the hydroxyl radical. Data plotted according to equation 6.

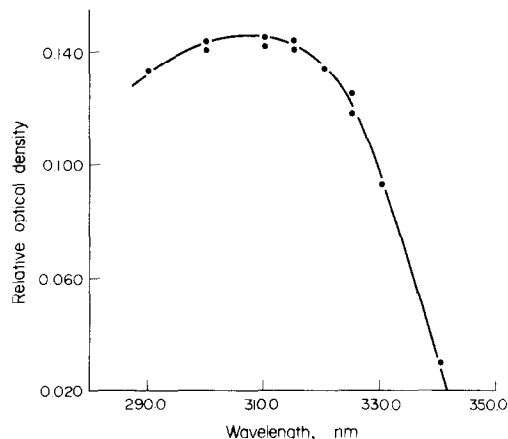


Fig. 2. Ultraviolet absorption band of the amygdalin hydroxycyclohexadienyl radical in aqueous solution containing nitrous oxide.

maximum of this band, which is undoubtedly due to the amygdalin hydroxycyclohexadienyl radical, resulting from OH addition to the phenyl group on amygdalin, is seen to be $310 \pm 3 \text{ nm}$. This is in accord with absorption spectra of various hydroxycyclohexadienyl radicals formed from other organic compounds [8]. There is a small contribution to this absorption spectrum by the cyclohexadienyl radical formed by H-atom addition [19] to the phenyl group in amygdalin. This constitutes a very minor contribution, since the yield of H-atom is very small compared with that of OH in N_2O solution (yield of H-atom is 0.5 molecule/100 eV; yield of OH is 5.5 molecules/100 eV and the molar extinction coefficients of the adduct radicals are comparable). No correction has been made in the spectrum of Fig. 2.

From the rate curve for the formation of the hydroxycyclohexadienyl radical, the value of k_1 for reaction 1 was determined independently. The formation of this radical in the amygdalin solutions was monitored between 300 and 320 nm. Plots of $\log(D_\infty - D_t)$, where D is the optical density, versus time gave a straight line showing first-order growth. The pseudo first-order rate constants, k_1 , for reaction 1 were obtained from the slopes of these lines (the concentration of amygdalin being very much larger than the concentration of OH radical). A plot of these values of k_1 versus the concentration of amygdalin has a slope equal to k_1 . These data are shown in Fig. 3. The slope of the line, k_1 , is equal to $(3.7 \pm 0.3) \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$. In determining this value for the addition rate constant, k_1 , no correction was made for the reaction of H-atom with amygdalin to yield the cyclohexadienyl radical. This approximation is permissible, since the yield of H-atom (as has been indicated) is only 9 per cent of the yield of OH in N_2O -saturated solution, and the effect on the rate constant, k_1 , is less than 9 per cent.

Since $k_2 = k_A - k_1$, k_2 for reaction 2 is found to be in the order of $4 \times 10^8 \text{ M}^{-1} \text{ sec}^{-1}$. The uncertainty in the value of k_2 is unavoidably large since it is obtained as a difference of two larger numbers, but the magnitude, $4 \times 10^8 \text{ M}^{-1} \text{ sec}^{-1}$, is consistent with previous values [8] for H-abstraction by the hydroxyl radical.

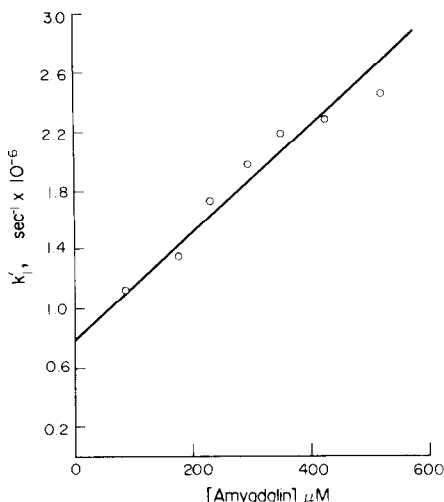


Fig. 3. Pseudo first-order rate constants, k'_1 , for the formation of the amygdalin hydroxycyclohexadienyl radical as a function of amygdalin concentration.

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