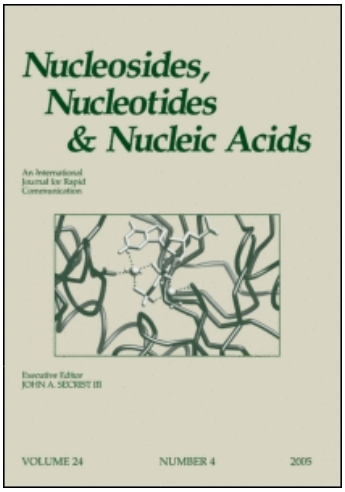

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Electrochemical Oxidations of Some Purine Nucleosides. Formation of Some Novel Purine Oligonucleosides

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ELECTROCHEMICAL OXIDATIONS OF SOME PURINE NUCLEOSIDES. FORMATION OF SOME NOVEL PURINE OLIGONUCLEOSIDES.

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ABSTRACT. The electrochemical oxidation of the purine nucleosides xanthosine and guanosine at carbon electrodes has been studied. The initial electrochemical event is a $1e^-$, $1H^+$ oxidation to give free radicals which undergo a series of follow-up chemical and electrochemical reactions which lead to formation of a new class of purine oligonucleosides.

INTRODUCTION

Electrochemical techniques provide powerful tools for investigating the oxidation-reduction (redox) chemistry of biological molecules.[1-4] For the past several years work in this laboratory has been concerned with the use of electrochemistry to elucidate the oxidation chemistry of purine bases.[5-19] In many instances it has been found that the oxidation pathways elucidated provide valuable insights into the peroxidase-catalyzed oxidations of purines.[10,20-24]

There have been very few studies of the electrochemical, chemical or biochemical oxidation chemistry of purine nucleosides and nucleotides. The electrochemical and peroxidase-catalyzed oxidations of 9-β-D-ribofuranosyluric acid proceed by very similar pathways[25,26] and by reactions which resemble those of the parent base uric acid. Recent studies of the electrochemical oxidations of xanthosine[27] and guanosine[28] reveal that the oxidation chemistry of these nucleosides is significantly more complex than that of the parent bases.

EXPERIMENTAL

Suppliers of chemicals and equipment used for electrochemical studies and spectral studies have been described in detail else-

where.[12,27,28] High resolution fast atom bombardment mass spectrometry (FAB-MS) was performed at the Midwest Center for Mass Spectrometry at the University of Nebraska, Lincoln, NE. Linear sweep and cyclic voltammograms were recorded at a pyrolytic graphite electrode (PGE) having an approximate surface area of 4 mm^2. All voltammetric measurements and controlled potential electrolyses were performed in solutions which were thoroughly deoxygenated with nitrogen. All potentials are referred to the saturated calomel reference electrode (SCE) at 25±3°C.

Electrochemical Oxidation of Xanthosine

Initial studies on the electrochemical oxidation of xanthosine(1) have been carried out at low pH because under these conditions some intermediates could be isolated and characterized along with several new oligomers. A cyclic voltammogram (CV) of 1 in aqueous trifluoroacetic acid (pH 2) shows one well-defined oxidation peak I_a followed by a second rather indistinct peak II_a at slightly more positive potentials (FIG. 1). Having scanned peak I_a several poorly defined bumps appear on the reverse sweep indicating the formation of reducible intermediates/products. On the second anodic sweep a new oxidation peak I_a' appears before peak I_a. The observed positive shift of the peak potential (E_p) for peak I_a with increasing sweep rate and concentration of 1 and the absence of a reduction peak coupled to peak I_a suggests that the peak I_a electrode reaction is irreversible.[29] At concentrations ≥1.0 mM peak I_a is largely diffusion controlled. The voltammetric n-value (number of electrons transferred per molecule of 1 electrooxidized) calculated from the irreversible peak I_a peak current[29] for 1.0 mM 1 was 1.2±0.2. The experimental n-value decreased to 1.0±0.2 using higher concentrations of 1. Based on the shift of E_p with pH for peak I_a (-0.086V per pH unit between pH 2-5) and measured values of αn_a (0.61±0.1) the number of protons transferred in the rate controlling irreversible reaction was calculated from Equation (1)[30] to be 1.0. Thus, under voltammetric conditions

$$dE_p/d(pH) = -\frac{0.059\ p}{\alpha n_a} \text{ at } 25°C \qquad (1)$$

the peak I_a electrooxidation of 1 is a $1e^-$, $1H^+$ reaction.

Preliminary controlled potential electrolysis experiments using several plates of pyrolytic graphite as the working electrode at

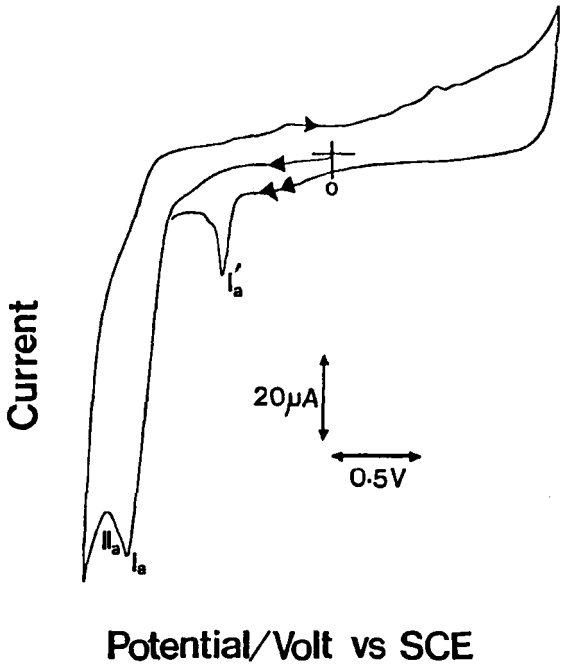

FIG. 1. Cyclic voltammogram at the PGE of 2.0 mM xanthosine in pH 2.0 trifluoroacetic acid. Sweep rate: 200 mVs^{-1}.

potentials corresponding to E_p for peak I_a of 1 revealed that products were formed which themselves were further electrooxidized. In order to obtain reliable coulometric n-values related to the primary electrooxidation of 1 very fast electrolyses ($\leq$5 min) were carried out using a reticulated vitreous carbon (RVC) electrode (7 cm x 2 cm diameter 100 ppi grade, Normar Industries, CA), which has a large surface area, dipping into 30 mL of pH 2.0 trifluoroacetic acid containing 1 at a potential (1.07V) which was considerably lower than E_p (1.25V). The amount of unoxidized 1 was determined by HPLC analysis of the resulting product solution. Using initial concentrations of 1 of 0.1, 0.5, 1.0, 1.5 and 2.0 mM experimental coulometric n-values of 2.1, 1.7, 1.5, 1.3 and 1.2 (±0.2), respectively, were measured. These results indicate that the nature of the overall electrode reaction changes as a function of the concentration of 1.

In order to electrooxidize 1 without appreciable electrolysis of oxidizable products and to minimize product decomposition such that

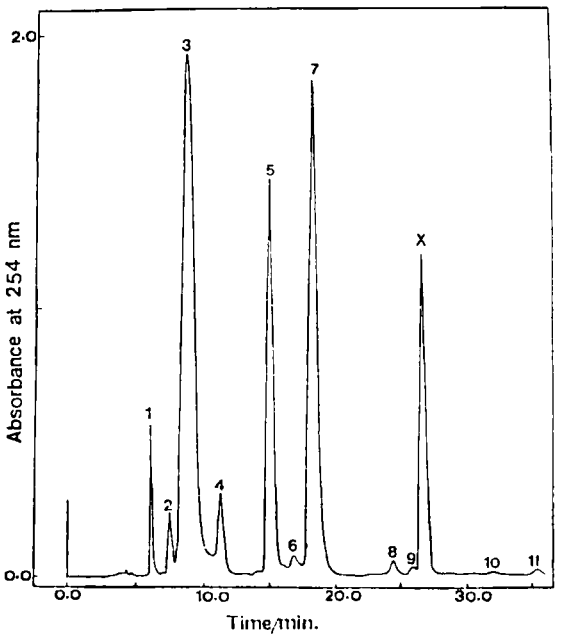

FIG. 2. Liquid chromatogram of the product mixture formed by electrooxidation of 1.8 mM xanthosine in pH 2.0 trifluoroacetic acid at an RVC electrode at 1.07V for 15 min at 4°C. Reversed phase column (Brownlee RP-18, 25x0.7 cm) with a mobile phase of H_2O:MeOH:MeCN(98:1:1,v/v) adjusted to pH 3.0 with trifluoroacetic acid. Flow rate: 2mL min^{-1} for 20 min then 4 mL min^{-1}. Volume of sample solution injected: 2.0 mL.

primary products could be isolated, the following procedure was employed. Electrooxidations of solutions of 1 (typically 2 mM) were carried out at an RVC electrode in pH 2 trifluoroacetic acid at 1.07 V at 0-4°C for no longer than 15 min. Product solutions were stored at -70°C and were melted only immediately before HPLC separation. A typical chromatogram showed 6 major peaks (HPLC peaks 1,2,3,4,5 and 7, FIG. 2). HPLC peak X is due to unoxidized 1. With decreasing initial concentrations of 1 electrooxidized, HPLC peaks 1 and 2 became much larger relative to the other peaks. Furthermore, if product solutions were allowed to stand at room temperature HPLC peaks 1 and 2 disappeared (ca. 15 min) without any corresponding increase in other major

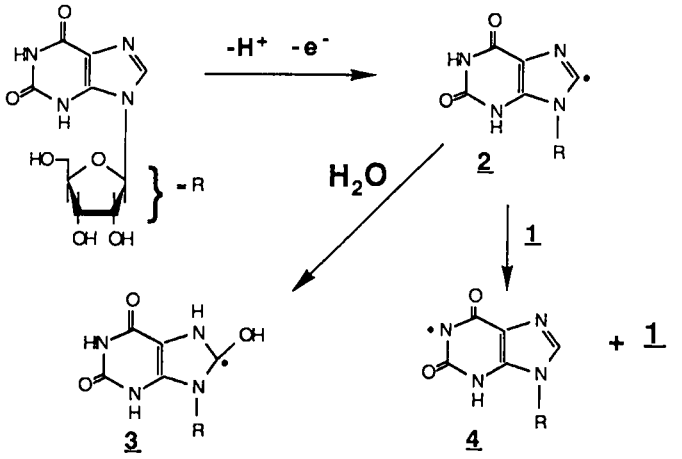

FIG. 3. Reaction pathway proposed for the initial peak I_a electrooxidation of xanthosine at pH 2.

peaks. HPLC peak 3 also decreased with time but with a corresponding increase in the height of HPLC peak 4.

The components eluted under HPLC peaks 1,2,3,4,5 and 7 were collected at -70°C, freeze dried and studied by UV spectrophotometry, IR spectroscopy, FAB-MS (thioerythritol/thiothreitol matrix) and ^{1}H-NMR spectroscopy.

Reaction schemes

Voltammetric measurements clearly indicate that the peak I_a electrooxidation of 1 is a $1e^-$, $1H^+$ reaction which must give a free radical. The initial species formed is believed to be the C(8)·xanthosyl radical 2 (FIG. 3). In view of some reaction products identified at least two more radicals must also be formed. Reaction of the primary C(8)·xanthosyl radical 2 with water gives the 8-hydroxy-xanthosyl radical 3. This is the predominant reaction when low concentrations of 1 are oxidized. At higher concentrations radical 2 can undergo a hydrogen atom abstraction reaction with 1 to give the N(1)·xanthosyl radical 4 (FIG. 3). Hydroxyradical 3 is further oxidized ($-1e^-1H^+$) to give 9-β-D-ribofuranosyluric acid (5, FIG. 4). The latter compound is more easily electrooxidized ($-2H^+-2e^-$) than 1 giving the quinonoid intermediate 6 which is rapidly attacked by water to give

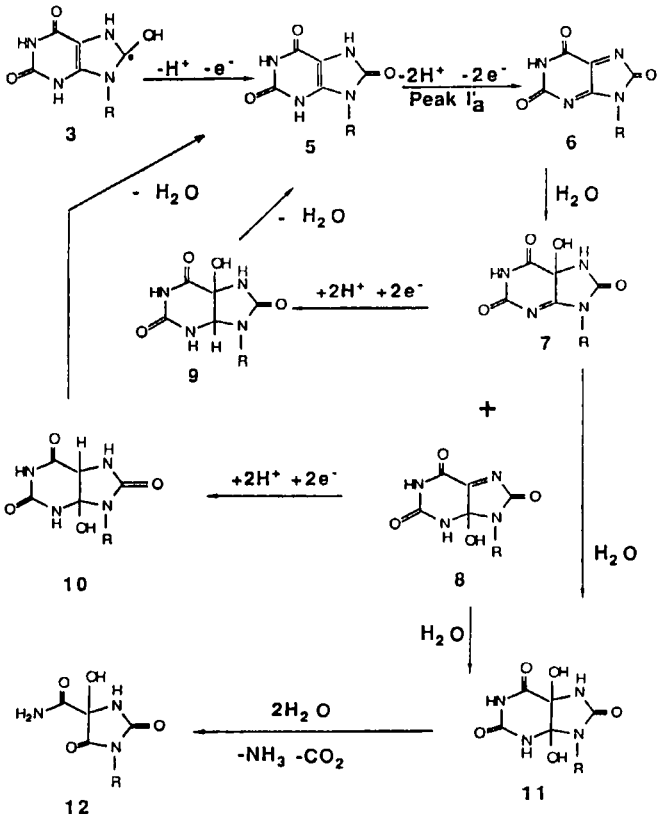

FIG. 4. Secondary oxidation of the 8-hydroxyxanthosyl radical 3 to 9-β-D-ribofuranosyluric acid 5 and hence to 5-hydroxyhydantion-5-carboxamide-3-riboside 12.

the isomeric tertiary alcohols 7 and 8. Compounds 7 and 8 (both MW = 316, $C_{10}N_{12}N_4O_8$, FAB-MS) are the intermediates responsible for HPLC peaks 1 and 2 (FIG. 2) and have identical UV spectra (λ_{max} = 268, 220nm at pH 3.0. They are also formed by electrooxidation of authentic 5 although the compounds responsible for HPLC peaks 3,4,5, and 7 are not. Intermediates 7 and 8 can be electrochemically reduced at negative potentials in CVs of 1 to form the dihydro compounds 9 and 10 which readily lose water to form 5. Peak I_a' observed on the second anodic sweep in CVs of 1 (FIG. 1) are due to oxidation of 5 formed in the latter reaction.[31] Further attack of water on tertiary alcohols 7 and 8 leads to diol 11 (MW = 334, $C_{10},H_{14}N_4O_9$, FAB-MS). Ring opening

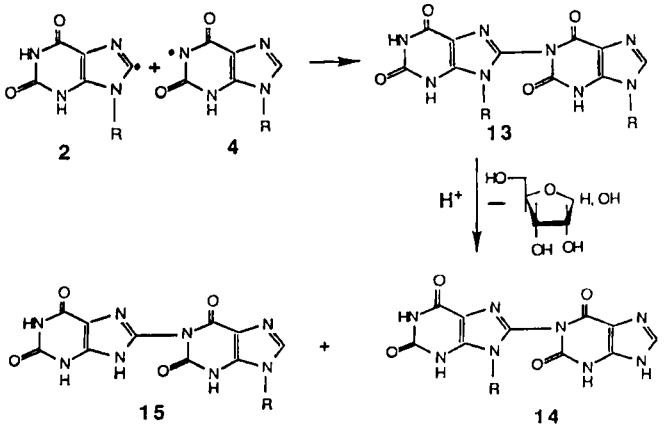

FIG. 5. Dimerization of C(8)· and N(1)· xanthosyl radicals to give 8-(1-xanthosyl)xanthosine(13) and then 8-(1-xanthosyl)-xanthine(15) and 1-(8-xanthosyl)xanthine(14).

and hydrolysis of diol 11 gives 5-hydroxyhydantoin-5-carboxamide-3-riboside 12 (FIG. 4).

The product eluted under HPLC peak 3 is a dimer (MW = 566, $C_{20}H_{22}N_8O_{12}$, FAB-MS) which is believed to be formed by coupling of the C(8)· and N(1)·xanthosyl radicals to give structure 13 (FIG. 5). 8-(1-Xanthosyl)xanthosine(13) is not very stable in acidic solution and, perhaps due to steric crowding, loses one ribose residue to give another dimer reponsible for HPLC peak 4 (FIG. 2). ^{1}H-NMR spectra indicate that two dimers (MW = 434, $C_{15}H_{14}N_8O_8$, FAB-MS) are eluted under HPLC peak 4 in which the C(8)-H and ribose moieties are in different environments. Thus, it is proposed that 8-(1-xanthosyl)-xanthine(15) and 1-(8-xanthosyl)xanthine(14) are formed.

HPLC peaks 5 and 7 (FIG. 2) are also due to dimers both of which have the same molecular weight and formula (MW = 584, $C_{20},H_{24}N_8O_{13}$, FAB-MS) and contain two xanthosine residues plus the elements of one molecule of water. Because of the tendency of these dimers to slowly decompose in water and DMSO it has not yet been possible to obtain definitive ^{1}H-NMR spectra. However, based largely on FAB-MS it has been tentatively concluded that the 8-hydroxyxanthosyl radical 3 reacts with either radical 2 to give 8-(8-hydroxyxanthosyl)xanthosine (16) or with radical 3 to give 1-(8-hydroxyxanthosyl)xanthosine (17) as shown in FIG. 6.

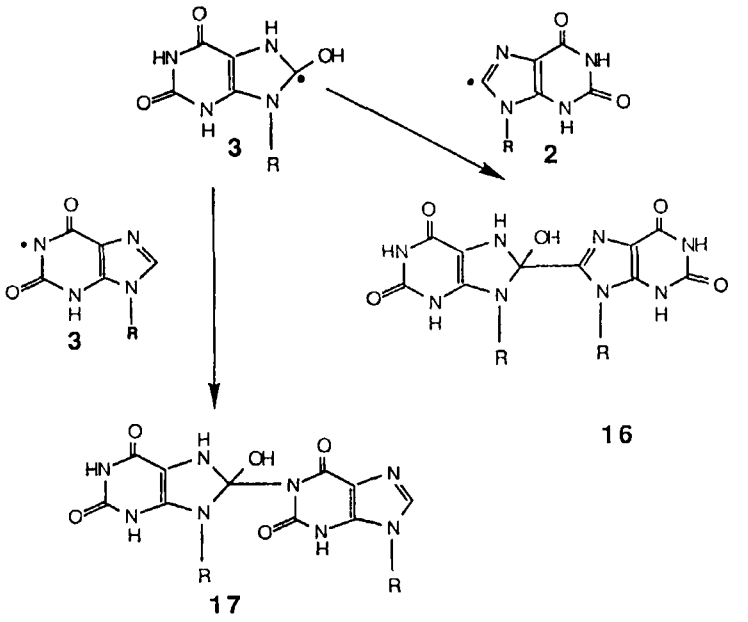

FIG. 6. Coupling of 8-hydroxyxanthosyl radical 3 with radicals 2 and 3 to form 8-(8-hydroxyxanthosyl)xanthosine (16) and 1-(8-hydroxyxanthosyl)xanthosine (17).

Electrochemical Oxidation of Guanosine

Between pH 2-11 guanosine (18) shows up to three, pH-dependent voltammetric oxidation peaks (I_a, II_a, III_a) at the PGE (FIG. 7). Having scanned through these peaks several reduction peaks appear on the reverse sweep and, on the second anodic sweep, two new oxidation peaks (I_a', II_a') appear at less positive potentials than peak I_a. Reduction peak I_c forms a quasi reversible couple with oxidation peak I_a'. Preliminary controlled potential electrolysis experiments at potentials corresponding to peaks I_a/II_a reveal that products are formed which are also electrochemically oxidized at these potentials. In order to minimize oxidation of these products rapid ($\leq$15 min), low temperature (4°C) partial electrolyses of 18 were performed. Coulometric n-values were obtained after measuring the amount of unoxidized 18 by HPLC analysis of the product solutions. In phosphate buffer pH 7.0 (μ = 0.5) electrolyses were performed at 1.20 V using several plates of pyrolytic graphite as the electrode material. Using

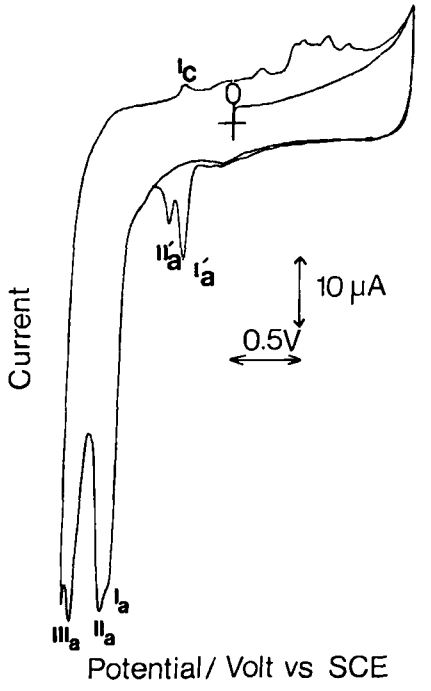

FIG. 7. Cyclic voltammogram at the PGE of 0.1 mM guanosine in pH 7.0 phosphate buffer (μ = 0.5). Sweep rate: 200 mVs^{-1}.

initial concentrations of 18 of 0.1, 0.2, 0.5, 1.0 and 1.5 mM coulometric n-values of 4.5, 3.2, 3.0, 2.6 and 2.5 (±0.2), respectively, were obtained. Thus, as was observed for electroooxidation of xanthosine, the initial concentration of guanosine greatly affects the observed n-values.

HPLC analysis of the product mixtures formed by peaks I_a/II_a electrolyses of 18 (>1 mM) in pH 7 phosphate showed at least 16 peaks. Compounds responsible for seven of these peaks have been isolated and partially or completely identified using FAB-MS, collisional activation FAB-MS and ^{1}H-NMR spectroscopy. Based upon electrochemical information and the products identified the primary peaks I_a/II_a electrooxidation of 18 appears to be a $1e^-$, $1H^+$ reaction giving the C(8)·guanosyl radical 19 (FIG. 8). In order to account for the various identified products radical 19 must either react with water to give the 8-hydroxyguanosyl radical 20 or with 18 in hydrogen atom

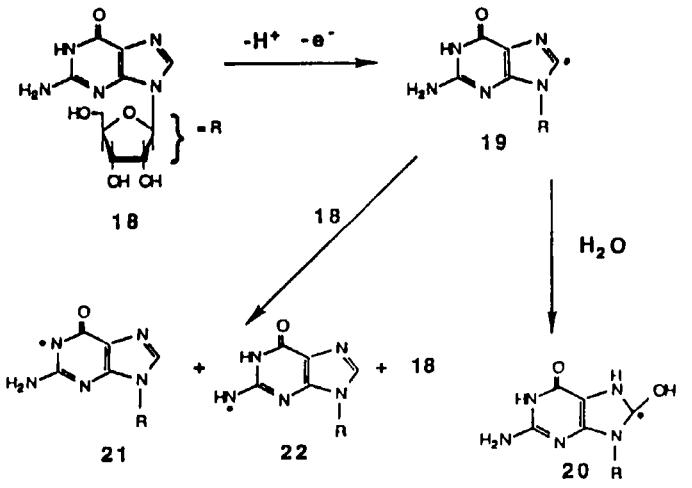

FIG. 8. Reaction scheme proposed for the primary peaks I_a/II_a electrooxidation of guanosine (18) to give 8-hydroxyguanosyl radical 20 and guanosyl radicals 21 and 22.

abstraction reactions to give the guanosyl radicals 21 and 22 as shown in FIG. 8. The former pathway is favored when low initial concentrations of 18 are oxidized. Further oxidation ($1e^-$, $1H^+$) of the 8-hydroxyguanosyl radical 20 give 8-hydroxyguanosine (23, FIG. 9). Since 23 is more easily oxidized than 18 it is immediately oxidized further. The electrochemical oxidation of 23 has not yet been studied in detail but, based on the known electrochemistry of the parent base 8-hydroxyguanine[19], it is very probable that 23 is oxidized ($-2e^-2H^+$) to quinonoid 24 (FIG. 9). It is 24 which is responsible for reduction peak I_c observed in CVs of 18 (FIG. 7) due to the quasi reversible reduction of 24 to 23. On the second anodic sweep in CVs of 18 peaks I_a' and II_a' are due to oxidation of 23. Quinonoid 24 is attacked by water to give tertiary alcohol 25 which rearranges to carboxylic acid 26 which in turn decomposes to products. Based upon earlier studies with 8-hydroxyguanine[19] it is likely that the major product of this reaction pathway is the riboside of 5-guanidinohydantoin.

A major peaks I_a/II_a electrooxidation product of guanosine is a dimer consisting of two guanosyl residues (MW = 564, $C_{20}H_{24}N_{10}O_{11}$,

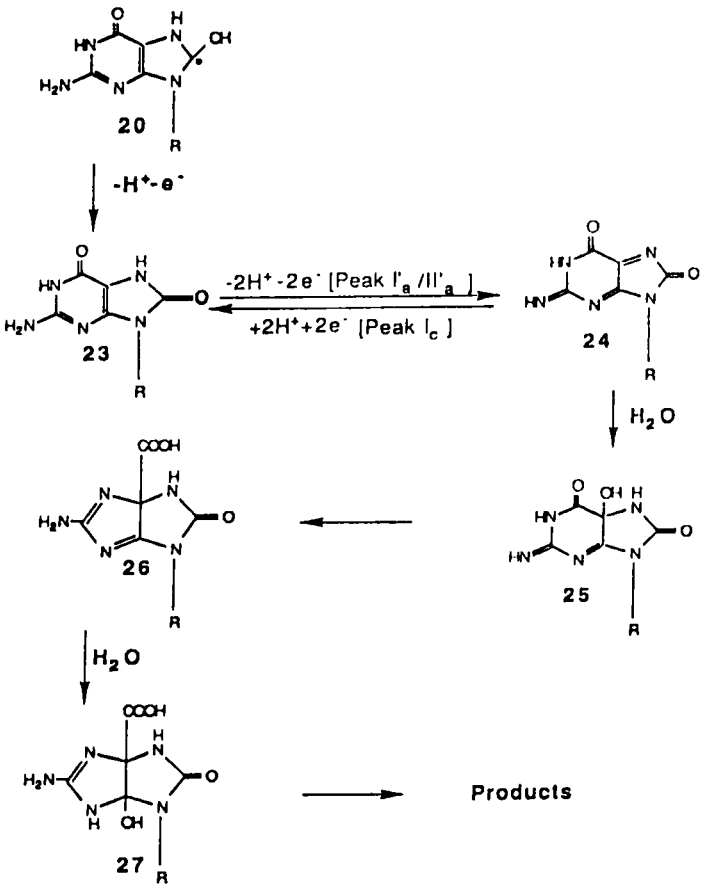

FIG. 9. Reaction scheme for electrooxidation of the 8-hydroxyguanosyl radical 20 to 8-hydroxyguanosine (23) and hence to products.

FAB-MS). ^{1}H-NMR spectroscopy indicates that this dimer is 8-(1-guanosyl)guanosine (28) which must be formed by coupling of guanosyl radicals 19 and 21 as shown in FIG. 10. Two additional isomeric dimers (MW = 580, $C_{20}H_{24}N_{10}O_{11}$, FAB-MS) are formed. FAB-MS and ^{1}H-NMR results indicate that these dimers are 30 and 31 (FIG. 11) in which two guanosyl residues are linked together with an oxygen bridge. A reasonable route to such dimers involves electrochemical oxidation ($-2H^+-2e^-$) of the 8-hydroxyguanosyl radical 18 to the oxyradical 29 which couples either with the N(1)· guanosyl radical to give 30 or with the exocyclic amino radical 22 to give 31 (FIG. 11).

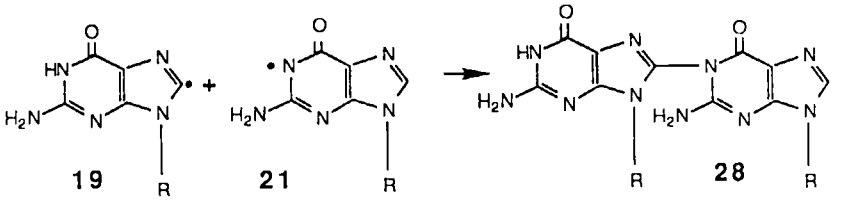

FIG. 10. Reaction scheme proposed for formation of 8-(1-guanosyl)-guanosine (28) by peaks I_a/II_a electrooxidation of guanosine.

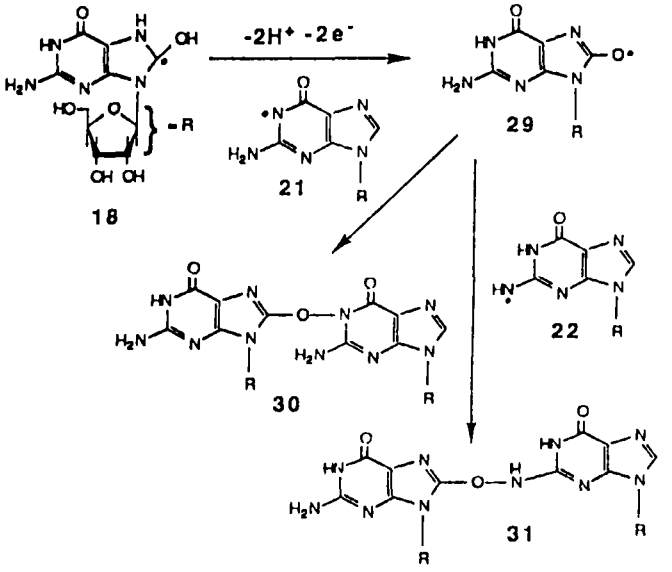

FIG. 11. Reaction scheme proposed for formation of 8-O-(1-guanosyl)-guanosine (30) and 8-O-(2-guanosyl)guanosine (31) upon peaks I_a/II_a electrooxidation of guanosine.

Two trimeric nucleosides have also been isolated following peaks I_a/II_a electrooxidation of 18 at pH 7. 8-(8-Guanosyl)-1-(1-guanosyl)-guanosine (34, FIG. 12) (MW = 845, $C_{30}H_{35}N_{15}O_{15}$, FAB-MS) could be formed by a number of routes. One feasible route involves dimerization of the N(1)·guanosyl radical 21 to give 1-(1-guanosyl)guanosine (32, FIG. 12). Dimer 32 has not been isolated and thus it is proposed that it is further oxidized either electrochemically ($-1e^-, -1H^+$) or chemically in a hydrogen atom abstraction reaction with radicals 19,

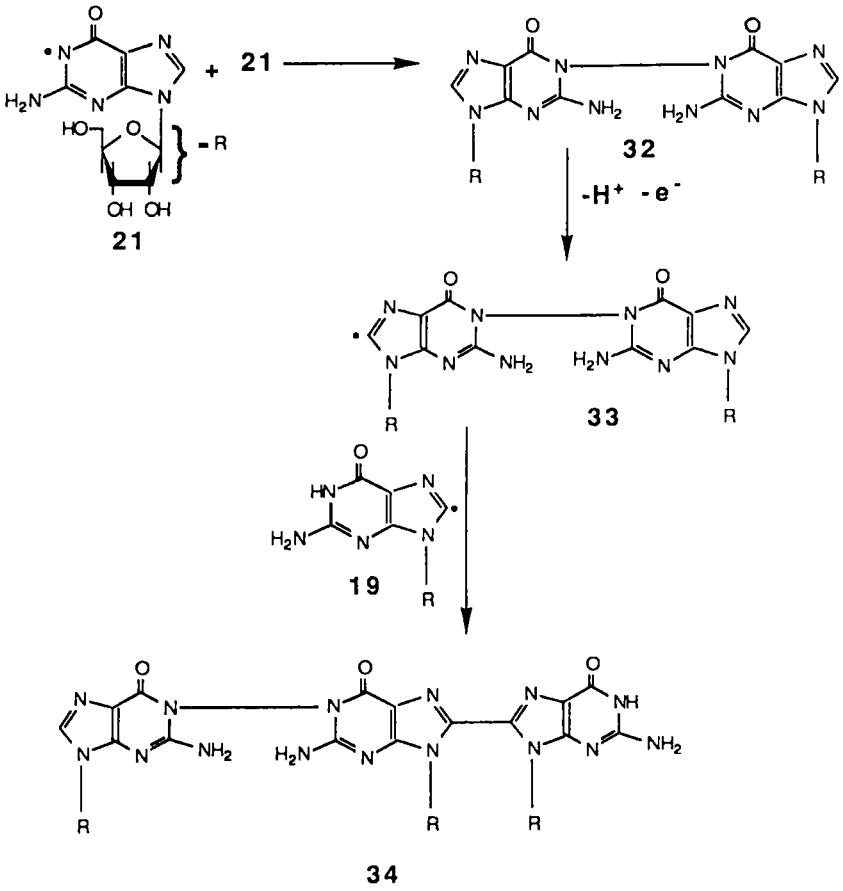

FIG. 12. Reaction scheme proposed for the formation of 8-(8-guanosyl)-1-(1-guanosyl)guanosine (34) upon peaks I_a/II_a electrooxidation of guanosine.

20, 21 or 22 to give the radical dimer 33. This can then couple with radical 19 to give the guanine trinucleoside 34.

A second trimeric nucleoside (MW = 862, $C_{30}H_{34}N_{14}O_{17}$, FAB-MS) has also been isolated in such low yield that it was not possible to obtain ^{1}H-NMR spectra. Based largely on FAB-MS data this trimer probably has structure 40 (FIG. 13). Trimer 40 differs from other identified oligomeric guanosine electrooxidation products in that one exocyclic amino group is absent. It is thus proposed that radical 22, which should also exist in the imino form 22a, is hydrolyzed to

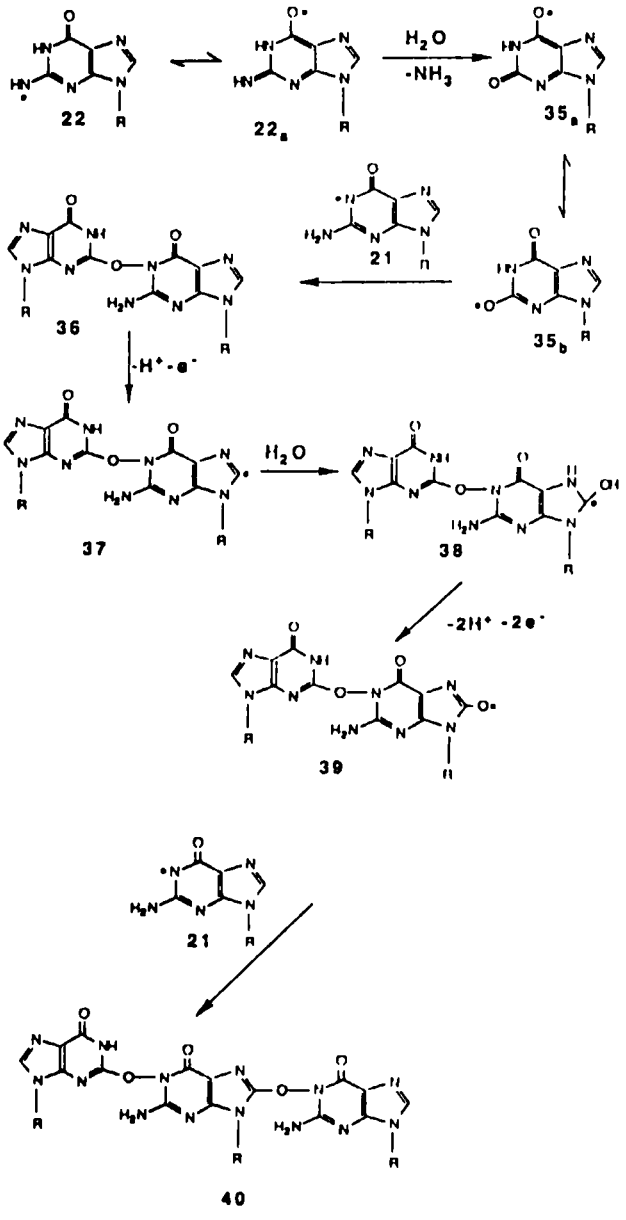

FIG. 13. Reaction scheme proposed to account for formation of 1-O-(2-xanthosyl)-8-O-(1-guanosyl)guanosine(40) upon peaks I_a/II_a electrooxidation of guanosine.

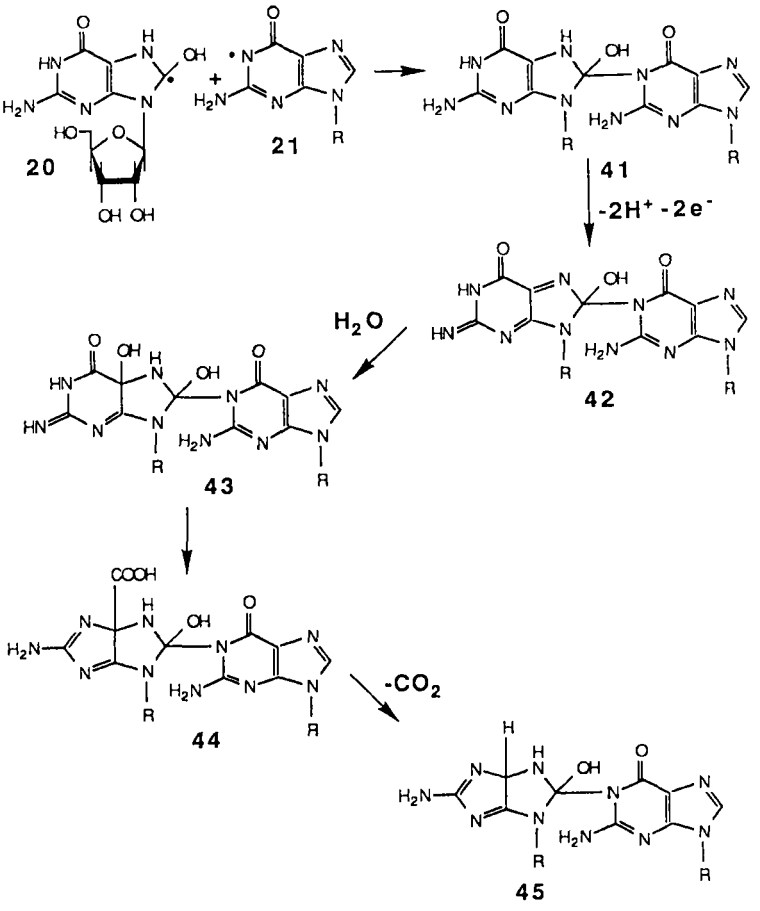

FIG. 14. Reaction scheme proposed for formation of 1,2-dihydro-3-hydroxy-3-(1-guanosyl)-4-β-D-ribofuranosyl-7-amino-2,4,6,8-tetraaza-4,7-diene-bicyclo-(3.3.0)-octane (45) upon peaks I_a/II_a electrooxidation of guanosine.

xanthosyl radicals 35a/35b (FIG. 13). Coupling of xanthosyl radical 35b with guanosyl radical 21 would give 2-O-(1-guanosyl)xanthosine (36). Electrochemical ($-1H^+-1e^-$) or chemical (-H•) oxidation of 36 would then give the radical dimer 37 which, following attack by water and further oxidation gives oxyradical 39. Coupling of radicals 21 and 39 then gives the isolated trimeric nucleoside 40 (FIG. 13). There are, of course, several alternative reaction pathways which could lead to trimer 40.

One additional dimeric compound (MW = 554, $C_{19}H_{26}N_{10}O_{10}$, FAB-MS) has also been isolated in very low yield. This dimer is not very stable in aqueous solution. Collisional activation FAB-MS on the pseudomolecular ion (MH^+) of this compound suggests structure 45 (FIG. 14). A reaction scheme is suggested in which the 8-hydroxyguanosyl radical 20 couples with the N(1)· guanosyl radical to give the hydroxylated dimer 41 (FIG. 14). Unlike other identified oligomers, 41 possesses an 8-hydroxyguanosyl residue and hence might be expected to be electrochemically oxidized ($-2H^+-2e^-$) to quinonoid dimer 42. The expected nucleophilic attack by water on 42 would give tertiary alcohol 43 and, by analogy with the known electrochemical oxidation pathway for 8-hydroxyguanine[19], this should rearrange to carboxylic acid 44. Decarboxylation of 44 would then lead to 1,2-dihydro-3-hydroxy-3-(1-guanosyl)-4-β-D-ribofuranosyl-7-amino-2,4,6-8-tetraaza-5,7-diene-bicyclo-(3.3.0)-octane 45 (FIG. 14).

The isolated guanine oligonucleosides are all electrochemically oxidizable. Thus, one or more of these species are probably responsible for the voltammetric oxidation peak III_a observed in CVs of guanosine (FIG. 7).

CONCLUSIONS

The reaction pathways described for formation of the various oligonucleosides formed upon electrochemical oxidations of xanthosine and guanosine must, at this time, be regarded as quite speculative. Furthermore, formation of a variety of reactive radicals in these oxidation processes would suggest that even larger oligomers should be formed. Indeed, liquid chromatography of product solutions obtained following electrochemical oxidation of both xanthosine and guanosine indicate the formation of a number of relatively minor products which have not yet been identified. It therefore appears likely that by careful control of experimental conditions it should be possible to design electrosynthetic methods to prepare a new class of purine oligonucleosides.

ACKNOWLEDGEMENTS

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