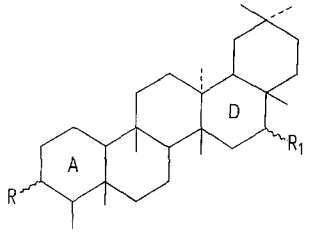

	R	R_1
(1)	=O	α-OH, β-H
(2)	=O	α-OAc, β-H
(3)	=O	α-OBz, β-H
(4)	=O	=O
(5)	α-OH, β-H	α-OH, β-H
(6)	α-H, β-OH	α-OH, β-H
(7)	=O	α-H, β-OH

hydroxy functions at C-3 and in ring D respectively. Oxidation of **1** with Corey's reagent[5] gave a diketone, m.p. 298–299 °C; $C_{30}H_{48}O_2$; M^+ 440; $[\alpha]_D^{18°}$ −35° ($CHCl_3$) identical with the synthetic dione (**4**) prepared earlier by Kikuchi et al.[6], and thus the hydroxy group was fixed at C-16. Evidence for its α-orientation was obtained by the reduction of **1** with Na/isoamyl alcohol to diol **5**, m.p. 288–290 °C; $C_{30}H_{52}O_2$; M^+ 444; $[\alpha]_D^{18°}$ −4.4° ($CHCl_3$), which was found to be identical with the one obtained by the reduction of diketone **4** under similar conditions. On the other hand, the observed dissimilarity between the diols **5** and **6**, [m.p. 275–277 °C; $C_{30}H_{52}O_2$; M^+ 444; $[\alpha]_D^{18°}$ −3° ($CHCl_3$), obtained by $NaBH_4$ reduction of (**1**)] is in conformity with the above stereochemical assignment and therefore the structure **1** is the complete representation of antidesmanol which is a C-16 epimer of pachysonol (**7**) isolated earlier from *Pachysandra terminalis*[6] (Buxaceae).

The antiinflammatory activity of the compounds was tested in mice against carrageenin-induced acute oedema[7]. Out of all compounds tested only n-tritriacontane at 100 mg/kg (p.o.) exhibited 50.6% activity. The diuretic activity was tested in rats[8] and only friedelin at 64 mg/kg (p.o.) showed 99% activity compared with chlorothiazide (125 mg/kg).

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Inhibition of nitrosamine formation by ascorbic acid: participation of free radicals in its anaerobic reaction with nitrite

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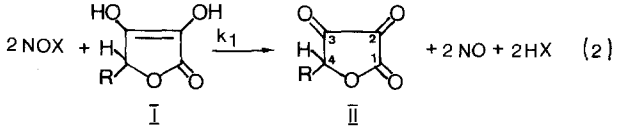
Summary. The participation of semiquinone free radicals during the reaction of ascorbic acid with acidified sodium nitrite has been demonstrated by ESR spectroscopy unambiguously for the first time. Scavenging of the nitrosating agent, reflected by the observed free radical concentration, unexpectedly occurs with scarcely varying efficiency over the pH range 0.1–4.5.

Nitrosamines (NOAs), among the most potent carcinogens currently recognised, are known to form intragastrically following the intake of nitrite together with various primary[1], secondary[2] or tertiary amines[3] (reaction 1).

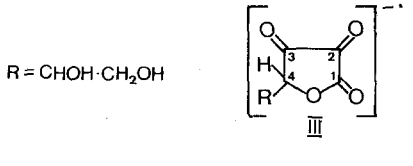
$$NOX + R_1R_2NH \rightarrow R_1R_2N{-}NO + HX \qquad (1)$$

Precursors of NOAs may occur in the human diet, originating form natural and man-made sources[4–7]. It is also known that naturally occurring phenolic substances in foodstuffs show an inhibitory effect on the NOA formation, while others such as chlorogenic acid and gallic acid effectively promote NOA formation[8,9]. Recent work however has demonstrated the possibly universal efficacy of ascorbid acid (**I**) in blocking the formation of nitroso compounds[10]. The mechanism is believed to involve the simple competitive scavenging of nitrosating agent (X=OH, H_2O^+ or NO_2 dependant on pH-value[11]) by **I** (reaction 2). Although reaction 2, originally described by Karrer[12], has more recently been the subject of some very detailed kinetic measurements differing mechanistic interpretations are possible[13].



I II

In view of its obvious importance to a better understanding of the NOA blockage mechanism we are undertaking ESR flow studies of reaction 2. Preliminary results of this work are presented here.



III

Experimental. Ascorbic acid, sodium nitrite and perchloric acid were all of the best commercially available purity and were used without further purification. Extreme

Table 1. ESR. spectral parameters of radical observed during oxidation of ascorbic acid

Method of preparation	pH-value	g factor	a_{H_4}	a_{H_5}	a_{H_6}	Literature reference No.
Photolysis	6.3	2.0052	1.75	0.06	0.17	16
Ti(*III*) H_2O_2		2.0054	1.7	-	0.17	17
Oxidation of (*I*) with dehydroascorbic acid	6.4	-	1.7	-	0.19	18
Oxidation of (*I*) with molecular oxygen	8.2	-	1.7	-	0.17	20
In situ radiolysis	4.0	2.00518	1.76	0.07	0.2	19
	0.5	2.00515	1.60	-	0.2	
Oxidation with nitrous acid	0.7	-	1.58	-	-	this work
	4.0	-	1.76	-	-	

Table 2. Observed ascorbic acid semiquinone radical concentration as a function of pH-value and substrate concentration. Solvent dioxane-water 3:5

Initial concentration Ascorbic acid (moles/$l \cdot 10^{-3}$)	Sodium nitrate (moles/$l \cdot 10^{-3}$)	pH-value	Radical concentration (moles/$l \cdot 10^{-6}$)	Range*
7.8	22	1.2	3.1	Taylor
5.4	47	1.5–2.0	4.5	Taylor
2.5	75	3.6	3.5	Ridd
0.4	96	4.6	1.0	Ridd

* The range names have been adopted after Dahn et al.[13]. They are defined as Taylor (pH 1.5–2.5) and Ridd (pH 3–5). The following rate laws are valid:

Taylor range: $v_T = k_3^{\circ} \cdot HNO_2^2 \cdot Asc^{\circ}$ ($k_3^{\circ} = 81$ moles^{-2} l^2 sec^{-1})

$v_T = k_3^{-} \cdot HNO_2^2 \cdot Asc^{-}$ ($k_3^{-} = 1.4 \cdot 10^{-5}$ moles^{-2} l^2 sec^{-1})

Ridd range: $v_R = k_2 \cdot HNO_2^2$ ($k_2 = 1.78 \cdot 10^{-2}$ moles^{-1} sec^{-1}) in dioxane-water (40:60) at 0 °C.

precautions (permanent argon blanketing of freshly boiled solvents and solutions) were undertaken to prevent aeration. As a result the effluent leaving the flow system contained <0.33 ppm oxygen. The ESR spectrometer and flow cell (volume 0.066 ml) have been described elsewhere[14,15]. In general combined flow rates were maintained at 0.4–0.7 ml/sec.

In common with the Swiss workers[13] we also encountered severe problems in maintaining a constant reaction pH value. In flow, pH values varying only by 0.1–0.2 units could be obtained, stationary however they sometimes varied by 1–1.5 units over a period of min. Complete reaction (see reaction 2) results in the loss of 3 H^+ ions. The radical concentration was determined by comparison with a standard solution of Mn (**II**).

Results and discussion. a) Nature of the free radical observed. From the resemblance between the experimental ESR signal here and the observed on photolytic[16], chemical[17–19] and radiolytic oxidation[20] of ascorbic acid we conclude that essentially the same radical is involved in all systems. Table 1 summarizes the ESR spectral parameters observed. Striking is the reduction of the C_4 proton coupling constant on lowering the pH-value from 4 to ca. 0.5, an effect previously commented on by Laroff et al.[20]. In accordance with these authors we suggest that a protonation equilibrium is involved, the low value of the C_4 hfs being explained by the low spin density at C_3 and the delocalised nature of the radical. Thus the ionised form of the radical observed probably has the structure **III** over the pH range 0.5–4.0 studied here.

b) General conditions of observation. Table 2 shows concentration and pH dependence of the radical yield for several initial substrate ratios. These data indicate that the most favourable conditions for observation of the radical intermediate are in the pH range 1.5–2.0 (Taylor range) using an approximately 10-fold excess of nitrite. This pH range approximates closely to that in the human stomach. Remarkable however, is the similarity in the magnitude of the radical concentrations in the Taylor and Ridd ranges despite their differing mechanisms and rate laws (Table 2). For example in typical reaction conditions employed here the rate of turnover of **I** in the Taylor range (v_T) will be 10^4–10^6 times that in the Ridd range (v_R). Superficially an attractive explanation for this may be the nearly identical contributions of the reactions

$$H_2NO_2^+ + Asc^{\circ} \rightarrow \text{Radical intermediate}$$

$$N_2O_3 + Asc^{-} \rightarrow \text{Radical intermediate}$$

as dominating processes in the relevant pH ranges[13]. The half-life of the reaction

$$2\,HNO_2 \rightarrow N_2O_3 + H_2O \quad (t_{1/2} \text{ ca. } 250 \text{ sec for } 10^{-1}\text{M } NaNO_2)$$

generating the nitrosating agent in the Ridd range, however, under these conditions appears much too long to allow appreciable conversion to this reagent within the time scale of the flow experiments (0.3–0.4 sec). It is thus unlikely that reactions based solely on N_2O_3 as nitrosating agent contribute exclusively to the high radical concentrations observed in the higher pH range. A more rapidly formed nitrite derived intermediate, not differentiated in the earlier work[13], may apparently also be participating in reactions leading to both free radical and dehydroascorbic acid (**II**) formation.

More recent work[21] postulates that the isomers of N_2O_3 ($ON \cdot NO_2$ and $ON \cdot ONO$) may be responsible for the vastly differing kinetics observed in some liquid phase nitrosations.

c) Concluding remarks on suppression of NOA formation by ascorbic acid. Although the overall 3rd order reaction between nitrous acid and ascorbic acid (ca. 100% Asc°; $k = 81$ moles^{-2} l^2 sec^{-1}) at pH value 1.5 occurs much more rapidly than with dimethylamine ($k = 0.031$ moles^{-2} l^2 sec^{-1})[21] and presumably other simple amines, effective

inhibition of NOA formation only occurs at ascorbic acid concentrations 2 or more times higher than the nitrite concentration i.e. in quantities 4 times the stoichiometric ones (reaction 2). Tannenbaum et al.[23] have also observed that some of the nitrite may not necessarily be active as nitrosating agent. A possible explanation may be associated, in analogy with the observed catalytic influence of phenols on dimethylamine nitrosation[22], with the formation, in a preequilibrium step, of the intermediate ascorbyl nitrite[13] as an 'activated' nitrosating agent. Such a species, although easily hydrolysed by acid, might survive sufficiently long to carry out nitrosations even in the presence of efficient inorganic nitrite scavengers. Until more evidence is available on this point it is therefore our opinion that even 'ascorbate protected' foodstuffs be more widely monitored for as yet undetermined NOAs possibly arising via this hitherto seldom considered route.

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The syntheses of spin labelled juvenoids

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Summary. 5 juvenoids labelled with stable nitroxyl radicals were synthesized and were shown to have morphogenetic effect on *Tenebrio molitor* L. and *Dysdercus cingulatus*.

Juvenoids labelled with tritium or ^{14}C are frequently used in studies of interactions of juvenile hormones and their analogs with biological receptors[1]. Spin labelled juvenoids have not yet been used in such studies and have never been described so far. In this communication we describe the preparation of juvenoids labelled with stable nitroxyl radicals to be used in biochemical studies. The synthetic routes which we followed in our syntheses are shown in the figure.

The syntheses started with readily available substrates such as geranylacetone (**1**), farnesol (**7**), and geraniol (**11**). A xylene solution of geranylacetone was refluxed in a Dean-Stark apparatus with 2-amino-2-methylpropan-1-ol in the presence of a catalytic amount of p-toluenosulfonic acid[2] to give 70% of oxazolidine **2**.

The oxazolidine **2** was next oxidized with m-chloroperbenzoic acid in diethylether to afford the nitroxyl derivative **3**; b.p. 180 °C/4 mm Hg[3], IR (film): 1375 cm^{-1}, 1250 cm^{-1}, 1160 cm^{-1}, 1118 cm^{-1}, EPR g-radical, $2\Delta B_{1S} = 1.37 \pm 0.01$ mT.

The dihydroderivative **6** was obtained in the same manner starting with dihydrogeranylacetone with overall yield 60%; b.p. 175 °C/2 mm Hg[3], IR (film): 1363 cm^{-1}, 1250 cm^{-1}, 1162 cm^{-1}, 1118 cm^{-1}, EPR g-radical, $a = 1.15 \pm 0.01$ mT, $2\Delta B_{1S} = 0.56 \pm 0.01$ mT. The nitroxyls **3** and **6** were obtained as stereoisomeric mixtures because the geranylacetone we used was a mixture of 2 isomers E/Z on double bond $C_5{=}C_6$ (65% E, 35% Z) and, moreover, the reactions with 2-amino-2-methylpropan-1-ol and m-chloroperbenzoic acid produced 2 racemic chiral centers at C-2 and C-9. A mixture of 4 isomers would be expected, but GLC analysis showed the presence of only 2. Since it was not possible to obtain any good NMR-spectrum (the presence in the molecule of an unpaired electron) we could not determine, at this stage of study, which stereoisomers were formed.

The compound **10**: b.p. 210 °C/3 mm Hg[3], IR (film): 1648 cm^{-1}, 1375 cm^{-1}, 1365 cm^{-1}, 1160 cm^{-1}, 1140 cm^{-1}, 890 cm^{-1}, EPR g-radical, $a = 1.57 \pm 0.01$ mT, $2\Delta B_{1S} = 0.41 \pm 0.01$ mT, was prepared by standard methods from commercial farnesol (Fluka). The ratio of $C_2{=}C_3$ double bond geometrical isomers was determine by GLC as 75% E and 25% Z.

The syntheses of ethers **15** and **16** were carried out starting with geranyl bromide and appropriate spin labelled alcohols **9** and **14** using a method similar to that described by Nilles et al.[4] followed by purification by TLC on silicagel. Compound **15**: decomposed during distillation at 210 °C, IR (film): 1670 cm^{-1}, 1375 cm^{-1}, 1362 cm^{-1}, 1175 cm^{-1}, 1075 cm^{-1}, 785 cm^{-1}, EPR g-radical, $2\Delta B_{1S} = 2.4 \pm 0.02$ mT; compound **16**: decomposed during distillation at 220 °C, IR (film): 1665 cm^{-1}, 1375 cm^{-1}, 1362 cm^{-1}, 1175 cm^{-1}, 1105 cm^{-1}, 840 cm^{-1}; EPR g-radical, $2\Delta B_{1S} = 1.12 \pm 0.02$ mT.

Preliminary biological tests of spin labelled compounds **3**, **6**, **10**, **15** and **16** (*Tenebrio molitor* L., *Dysdercus cingulatus* L.) revealed that the highest JH-activity was exhibited by the nitroxyls **3** and **6**. Geranyl ether **16** has only weak activity and **15** is not active (table). The details of these investigations will be published in a separate paper.